REPORT

of

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., Inc.

1983

Organization and Policy

The Council for Tobacco Research–U.S.A., Inc. is the sponsoring agency of a program of research into questions of tobacco use and health. It is the outgrowth of an organization formed early in 1954 by tobacco manufacturers, growers and warehousemen. Research support has been mainly through a program of grants-in-aid supplemented by contracts for research with institutions and laboratories. The Council does not operate any research facility.

The Scientific Advisory Board to The Council meets regularly to evaluate applications for research support, judging them solely on the basis of scientific merit and relevance.

The Council awards research grants to independent scientists who are assured complete scientific freedom in conducting their studies. Grantees alone are responsible for reporting or publishing their findings in the accepted scientific manner – through medical and scientific journals and societies.

WILLIAM D. HOBBS
Chairman

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LIAM D. HOBBS
Chairman

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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., Inc.
900 Third Avenue, New York, N.Y. 10022

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as of December 31, 1983

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Introduction

In 1957, three years after its formation, the Council for Tobacco Research printed and distributed the first of what became annual reports to contain abstracts of papers that had been published in various scientific media with acknowledgement of Council support. There were only 16 abstracts in that long-ago document. It had, understandably, taken time for organization, review and approval of grant applications by the Council's Scientific Advisory Board, research to be completed, and manuscripts to be prepared, submitted and accepted for publication.

By mid-1967, the total number of scientific publications acknowledging Council support was 616, and ten years later the figure had risen to 1,507. In the 1983 annual report, 186 scientific publications are abstracted, the largest number in any one year. This figure brings to at least 2,354 the total number of scientific papers acknowledging Council support published since the Council began supporting research by independent scientists into smoking and health.

These publications are an invaluable source of information and reference for researchers everywhere and, therefore, represent a major contribution to the scientific literature. They also can be considered a symbol of a research program that has become the most extensive effort of its kind in the world.

Last year, the Council increased the annual budget for its research program and maintained the increase for this year. This action is indicative of the Council's dedication to the program.

As of the end of 1983, the Council's 30-year program has provided some $83,000,000 to 497 investigators for 865 original projects in 279 medical schools, hospitals and research institutions.

Abstracts of Reports

Following are abstracts, approved by the authors, of reports on new research acknowledging support from The Council that have appeared in scientific journals since publication of the 1982 Report. The name of the grant recipient is in italics.

The abstracts are grouped under these headings: I. Cancer-Related Studies, II. The Respiratory System, III. Heart and Circulation, IV. Neuropharmacology and Physiology, V. Pharmacology and Biochemistry, VI. Immunology and Adaptive Mechanisms, VII. Epidemiology.

I. *Cancer-Related Studies*

EVOLUTION OF ACTIVATION-DETOXIFICATION ENZYME PATTERNS DURING PRECARCINOGENESIS IN SYNERGISM: 3-METHYLCHOLANTHRENE AND DIMETHYLNITROSAMINE

The working hypothesis of this study is that pulmonary syncarcinogenesis between polycyclic hydrocarbons and nitrosamines is due to the reciprocal effects of the agents on their own and on each other's metabolic pathways. To test this, the effects of 3-methylcholanthrene (3MC) and dimethylnitrosamine (DMN), administered singly or in combination at chronic carcinogenic doses, on the evolution of activity patterns involved in the metabolism of DMN and polycyclic hydrocarbons were studied in the liver and lung of Sprague-Dawley rats and Swiss mice. The data accumulated in the rat studies suggest that in the first stage of the process, at 7 weeks, there occurs a decreased activation and increased detoxification of 3MC; this is followed around 15 weeks by a great increase of activation concomitant with decreasing detoxification. The net result of these activity changes may be a high concentration of activated intermediates of 3MC in the lung. Also, the moderate enhancement of activity noted with DMN-d in the lung of rats (administered DMN or DMN + 3MC) would similarly lead to increase in the reactive metabolite(s) of DMN. This and other data suggest that the previously observed pulmonary syncarcinogenesis between 3MC and DMN in rats is probably due to increase of reactive metabolites of *both* 3MC and DMN. In the mouse liver, on the other hand, a combination of initial increase of epoxide hydrase and UDP-glucuronyl transferase activity at 5 weeks, together with decrease of AHH activity at 10 weeks, suggests a decrease of activated hydrocarbon metabolites available for binding to macromolecules. The overall data presented here are consistent with previous observations that activated metabolites originating from liver are unlikely to be contributors in DMN + 3MC pulmonary syncarcinogenesis.

Lai, D. Y., Bryant, G. M., Myers, S. C., Woo, Y. T., Argus, M. F., and *Arcos, J. C.*

Journal of Cancer Research and Clinical Oncology 103:227-240, 1982.

From the Department of Medicine, Tulane University Medical Center, New Orleans.

COMPARATIVE PULMONARY TUMORIGENESIS IN DBA/2J and C57Bl/6J MICE BY ADMINISTRATION OF 3-METHYLCHOLANTHRENE AND DIMETHYLNITROSAMINE SINGLY AND COMBINED

In this pulmonary tumorigenesis study, C57Bl/6J mice which are inducible for both hepatic and pulmonary aryl hydrocarbon hydroxylase (AHH), and DBA/2J mice,

which are noninducible for hepatic AHH but moderately inducible for pulmonary AHH, received dimethylnitrosamine (DMN) i.p., or methylcholanthrene (MCA) orally, or a combination of both agents, for 10 weeks; the animals were observed for an additional 26 weeks. The lung lesions induced here were alveologenic tumors and adenomatosis. Alveologenic tumors were induced in a much larger number of DBA/2J mice than in C57Bl/6J mice. If, however, the sum of alveologenic tumors and adenomatous nodules is considered and is expressed per lung lesion bearing mouse, then these ratios are for the control, MCA, DMN and MCA + DMN groups: 2.7, 1.5, 3.5, and 5.4 in the DBA/2J mice and 1.0, 1.3, 2.7, and 3.6 in the C57Bl/6J mice, respectively. This suggests a relatively greater susceptibility of the C57Bl/6J strain if the ratios are compared to the respective control values. This greater susceptibility of the C57Bl/6J is best seen by comparing the percent increase of the ratio for the MCA + DMN groups; the net increase is 100% for DBA/2J and 260% for C57Bl/6J.

Argus, M. F., Hoch-Ligetti, C. and *Arcos, J. C.*

Neoplasma 29(5):527-534, 1982.

From Seamen's Memorial Research Laboratory, U.S. Public Health Service Hospital, New Orleans, and the Department of Medicine, Tulane University Medical Center, New Orleans.

INCREASED LEVEL OF PROLACTIN GENE SEQUENCES IN BROMODEOXYURIDINE TREATED GH CELLS

The thymidine analogue, 5-bromodeoxyuridine (BrdUrd), has been found to influence several differentiated functions in various eukaryotic cells. Earlier studies have shown that BrdUrd incorporates into cell DNA replacing thymidine, and that induction of prolactin (PRL) synthesis by the drug seems to be dependent on DNA synthesis and such base substitutions. In the results reported here, it was seen that the 5-bromodeoxyuridine-resistant (BrdUrd^r) derivative ($F_1BGH_1 2C_1$) of prolactin nonproducing (PRL$^-$) rat pituitary tumor cell-subclone $GH_1 2C_1$, synthesizes PRL in the presence of the drug. Analysis of nuclear RNA isolated from BrdUrd treated $F_1BGH_1 2C_1$ cells demonstrated several high molecular weight RNA_{PRL} sequences, similar to those observed in the nuclear RNA fraction of PRL producing (PRL$^+$) GH_3 cells. No such RNA_{PRL} sequences could be detected in nuclear RNA fraction of untreated $F_1BGH_1 2C_1$ cells. PRL sequences in the genome of GH_3 (PRL$^+$), $GH_1 2C_1$ (PRL$^-$) and $F_1BGH_1 2C_1$ (PRL$^-$, BrdUrd^r, GH cells could be identified by blot analysis in 4.8-5.2kb fragment of restriction endonuclease, Hind III digested DNA. Both PRL$^+$ and PRL$^-$ cells seem to have approximately the same level of PRL gene sequences in total cell DNA. However, Hind III digested DNA of BrdUrd treated $F_1BGH_1 2C_1$ cells revealed the presence of significantly higher levels of PRL gene sequences, in comparison to that observed in total DNA of untreated cells. The increased level of PRL gene sequences was dependent on the period of drug treatment and a parallel increase in the cytoplasmic RNA_{PRL} sequences was also observed.

Biswas, D. K. and Hanes, S. D.

Nucleic Acids Research 10(13):3995-4008, 1982.

Other support: National Cancer Institute.

From the Laboratory of Pharmacology, Harvard School of Dental Medicine, and the Department of Pharmacology, Harvard Medical School, Boston.

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GENETIC AND EXTERNAL REGULATION OF NATURAL KILLER CELL CYTOTOXICITY IN MICE

Natural killer (NK) cells, which constitute one of the natural immune mechanisms postulated to play a role in defense against neoplastic development and metastasis, are regulated by a number of genes controlling not only NK cell number but also their lytic ability. In many strains of mice, the normal level of NK activity diminishes with age. In this paper, studies on 11 inbred and recombinant inbred strains of mice, and some of their hybrids, are presented. They demonstrate that the gradual loss of normal NK activity with aging is also accompanied by a loss in the ability to respond to interferon induction with augmented NK activity. Further, evidence is provided that differences in genotype can produce dramatic differences in NK activity in mice chronically exposed to a virus in a contaminated mouse colony. Finally, a genetic analysis of the effect of an oncogenic agent, pristane, on NK activity is presented. Pristane, which induces plasmacytomas in some strains of mice, such as the BALB/c, suppresses not only T cell and B cell responses but also NK activity. It is now shown that there are significant strain differences in the effect of pristane on NK activity, as well as differences in recovery from that depression. Particularly, studies of hybrids reveal that the genetic mechanisms responsible for the pristane-induced loss of NK activity in the BALB/c and in the C57BL strains are not the same.

Blair, P. B. et al.

In: Crispen, R. G. (ed): *Cancer: Etiology and Prevention*, New York: Elsevier Science Publishing Co., Inc., 1983, pp. 191-198.

Other support: National Cancer Institute, American Cancer Society and research funds of the University of California.

From the Department of Microbiology and Immunology, and the Cancer Research Laboratory, University of California, Berkeley.

INHIBITOR CELLS IN SPLEENS OF MICE WITH LOW NATURAL KILLER ACTIVITY

In surveys of various inbred strains of mice for natural killer (NK) cell activity, striking variations in normal responsiveness have been noted. For example, BPS mice normally have very low spleen cell NK activity. However, their NK activity can be significantly augmented with the use of an interferon inducer, which suggests that this basic low reactivity is not a result of low numbers of precursor cells or defects in the lytic mechanism. We report that BPS spleens contain suppressor cells, as evidenced by the ability of BPS spleen cells to inhibit *in vitro* the NK activity of spleen cells from high-responder strains of mice. Cells responsible for the suppression are located in the dense fraction after separation in Ficoll-Hypaque and are nylon wool-adherent. Kinetic studies indicate that the mechanism of inhibition is not merely competition for target-binding sites. Although these suppressor cells can act on mature NK effectors *in vitro*, their activity *in vivo* may be more complex, since mature BPS NK effectors are not revealed when the suppressors are removed.

Blair, P. B., Staskawicz, M. O. and Sam, J. S.

Journal of the National Cancer Institute 71(3):571-577, 1983.

Other support: National Cancer Institute, American Cancer Society and research funds of the University of California.

From the Department of Microbiology and Immunology, and the Cancer Research Laboratory, University of California, Berkeley.

MULTIPLE, IMMUNOIDENTICAL FORMS OF PHENOBARBITAL-INDUCED RAT LIVER CYTOCHROMES P-450 ARE ENCODED BY DIFFERENT mRNAS

The work reported here involved *in vitro* translation studies using Long-Evans and Holtzman rats. In this study, one colony of Long-Evans rats exhibited only cytochromes P-450b_{1F} and P-450*e* among these immunorelated enzymes; whereas, one colony of Holtzman rats was characterized by cytochromes P-450b_{1H} and P-450*e*. After phenobarbital treatment, hepatic poly (A)$^+$-mRNA was isolated from these groups of rats and translated *in vitro*. The ^{35}S-labeled products were immunoisolated with antibody to cytochrome P-450b_{1F} and analyzed by two-dimensional gel electrophoresis. The results indicated that the products synthesized *in vitro* correspond exactly to the two particular forms of the enzyme that characterize liver microsomes from each of these groups of rats. It is concluded that different structural genes encode these immunorelated forms of cytochrome P-450 and that significant post-translational processing of their polypeptide products does not occur *in vivo*.

Walz, F. G., Jr., Vlasuk, G. P., Omiecinski, C. J., *Bresnick, E.*, Thomas, P. E., Ryan, D. E., and Levin, W.

The Journal of Biological Chemistry 257(8):4023-4026, 1982.

Other support: National Institutes of Health and the National Science Foundation.

From the Department of Chemistry, Kent State University, Kent, OH; the Department of Biochemistry, University of Vermont School of Medicine, Burlington; and the Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche, Inc., Nutley, NJ.

BINDING OF BENZO[α]PYRENE AND INTRACELLULAR TRANSPORT OF A BOUND ELECTROPHILIC BENZO[α]PYRENE METABOLITE BY LIPOPROTEINS

In this biochemical study, noncovalent uptake of [^{3}H]benzo[α]pyrene was quantitated for fractions collected from the effluent of a liquid chromatographic separation of human serum and was found to directly correlate with the lipoprotein concentration. An electrophilic benzo[α]pyrene metabolite, [^{3}H]*trans* 7,8-dihydrodiol-9,10-epoxybenzo[α]pyrene, non-covalently associated with low density lipoproteins was transferred to human lymphocytes *in vitro* and bound acid-precipitable nucleic acids of the lymphocytes as a function of time. Benzo[α]pyrene metabolite binding to lymphocyte DNA was demonstrated by means of CsCl density gradient analysis. Nonmitogen-stimulated lymphocytes exposed to very low concentrations of carcinogen in the presence of low density lipoprotein demonstrated [^{3}H]thymidine incorporation; without the concomitant addition of low density lipoprotein the low concentrations of carcinogen did not stimulate [^{3}H]thymidine incorporation. Overall, the data presented here, which indicate that the efficiency of carcinogen binding to, or association with, lipoproteins is dependent on lipoprotein concentration, are suggestive of a significant role for lipoproteins in the cellular interaction of lipophilic carcinogens with human cells.

Busbee, D. L. et al.

Carcinogenesis 3(10):110

Other support: American Research Grant.

From the Genetics Cent University, Denton.

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Annals of Clinical and La

Other support: U.S. Publ

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Busbee, D. L. et al.

Carcinogenesis 3(10):1107-1112, 1982.

Other support: American Cancer Society and a North Texas State University Faculty Research Grant.

From the Genetics Center, Department of Biological Sciences, North Texas State University, Denton.

ENHANCED VIRAL TRANSFORMATION OF SKIN FIBROBLASTS FROM NEUROFIBROMATOSIS PATIENTS

Cultured skin fibroblasts (SF) from patients with certain autosomal dominant precancerous states exhibit increased sensitivity to oncogenic virus transformation. The data presented show the occurrence of enhanced viral transformation of cultures of SF established from patients with neurofibromatosis (NF). In this study, cultures of skin fibroblasts from persons with and without clinical NF in families in which the disorder had been diagnosed were examined for transformability by Kirsten murine sarcoma virus. The viral transformation results were compared with those obtained with SF cultures initiated from controls in families without history of any disorder with an hereditary component, or cancer. The results show that 63 percent of cultures from patients with clinical NF were transformed, compared with seven percent of control cultures. Cultures of skin fibroblasts from persons without the classical features of NF, but in families in which the disorder had been recognized, also exhibited a relatively high transformation rate, since 75 percent were transformed. This work indicates that neurofibromatosis can be included among other hereditary disorders in which enhanced transformability of cultures of SF by an oncogenic virus may be demonstrated.

Bidot-Lopez, P. and *Frankel, J. W.*

Annals of Clinical and Laboratory Science 13(1) 27-32, 1983.

Other support: U.S. Public Health Service.

From the Tampa Branch Laboratory, Department of Health and Rehabilitative Services, Tampa, FL.

DUCT, EXOCRINE, AND ENDOCRINE COMPONENTS OF CULTURED FETAL MOUSE PANCREAS

Since intercellular interactions may be important in carcinogenesis of the pancreas, the growth of at least three kinds of epithelial cells, *i.e.*, duct, acinar, and centroacinar in the same culture could be important. The purpose of the paper presented here was to characterize pancreas cells cultured on a pigskin substrate and to describe the optimal culture conditions. For this study, a total of 20 to 22 days postcoitum mouse fetal pancreas organ bits were cultured on the dermal surface of irradiated pigskin as a substrate. The medium used for long term culture consisted of Eagle's Minimum Essential Medium with the addition of 10% bovine serum, 0.02 U/ml insulin, 0.25 μg/ml glucagon, 3.63 μg/ml hydrocortisone, 100 μg/ml soybean trypsin inhibitor or 10^{-8} M atropine. When the medium lacked trypsin inhibitor or atropine but contained the three hormones, the pigskin support began to be destroyed after 2 to 4

wk in culture. Thereafter, the cultured cells could not grow and survive on the digested pigskin. When 10^{-6} M atropine was added to the medium, amylase secretion from cultured cells and destruction of pigskin were inhibited completely but pancreas cells could not grow or survive. In contrast, 100 μg/ml soybean trypsin inhibitor or 10^{-4} M atropine permitted cell growth and amylase secretion from the cultured acinar cells, and prevented the destruction of pigskin. Under these conditions, pancreas cells migrated and/or grew from the organ bits onto the surface of the pigskin dermis and organoid aggregations formed. Hydrocortisone was needed to permit growth for more than 2 wk. Glucagon and insulin had additive effects. Light and electron microscopic observations indicated the culture of at least five kinds of cells, *i.e.*, duct, acinar, centroacinar, endocrine, and mesenchymal. The majority of cultured cells were duct cells and acinar cells; there were few mesenchymal cells. Mouse panceas cells were cultured for at least 12 wk by this method.

Hirata, K., Oku, T. and *Freeman, A.E.*

In Vitro 18(9):789-799, 1982.

Other support: National Cancer Institute and the American Cancer Society.

From the Center for Neurologic Study, San Diego, CA.

POPULATION DISTRIBUTION OF PLACENTAL BENZO(α)PYRENE METABOLISM IN SMOKERS

Human placental microsomes isolated from term placentas derived from nonsmoking women and women smoking one to 40 cigarettes a day were analyzed for the metabolism of benzo(α)pyrene (BP) measured as various metabolites by high pressure liquid chromatography (HPLC) and/or as aryl hydrocarbon hydroxylase (AHH) activity. Results show that AHH activity was several times higher in smokers than in nonsmokers. Regression analysis on 13 different placental tissues from women smoking from one to 40 cigarettes demonstrated a high correlation between AHH activity (or the formation of BP phenols resolved by HPLC) versus the formation of the procarcinogenic BP-7,8-diol. Subsequent studies on placentas derived from 67 women who smoked 10 to 40 cigarettes per day demonstrated a definite dose-response relationship between AHH activity and the number of cigarettes smoked/day. The dose-response curve was sigmoidal in shape; however, when the data were plotted on a semi-log scale the curve assumed a linear shape, reaching saturation of AHH induction between 20 to 25 cigarettes/day. While mean AHH activity was dependent upon the number of cigarettes smoked/day, considerable interindividual variability in AHH (ranging more than 1,000-fold in some cases) was observed among individuals with comparable smoking histories. In further studies, population distribution suggested clustering of the population in the low-AHH-activity region while cord-blood thiocyanate analysis and twin studies suggested that genetic factors contributed to a major portion of the inter-individual variability in AHH activity observed among smokers.

Gurtoo, H. L. et al.

International Journal of Cancer 31(1):29-37, 1983.

Other support: National Institutes of Health and the American Cancer Society.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, and the Department of Pediatrics, University of Colorado School of Medicine, Denver.

THE PREPARATION OF [^{35}S]METHIONINE

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THE PREPARATION OF [^{35}S] HOMOCYSTEINE THIOLACTONE FREE OF [^{35}S]METHIONINE

A novel, simple and fast procedure for recovering methionine-free [^{35}S] homocysteine thiolactone following its synthesis is described in this methodology paper. As reported here, after [^{35}S]methionine was synthesized, the product was isolated from methionine by taking the mixture up in 200 µl of absolute methanol and placing the resulting solution on a column containing 1 ml of M.C.B. dry chromatographic alumina. The column was eluted with methanol, and the purity of the product was then determined by amino acid analysis. Thin layer chromatography was used as a second method to determine if the synthesized homocysteine was methionine-free. Results show that the yield of the alumina-column purified [^{35}S]homocysteine is 5-10% but, importantly, it provides a method for the complete separation of [^{35}S]homocysteine thiolactone from unreacted [^{35}S]methionine. The methionine is held strongly by the alumina through its carboxyl group and is not eluted by methanol. Homocysteine thiolactone, lacking a carboxyl group, is eluted very rapidly. This rapid and easy purification of the thiolactone, free of salts, makes a very convenient method for the preparation of methionine-free label for use in biological systems. This radioactive compound should be of important use as a tracer in studying the important reactions of homocysteine with no ambiguity due to contaminating methionine.

Stern, P. H., Mecham, J. O. and *Hoffman, R.M.*

Journal of Biochemical and Biophysical Methods 7:83-88, 1982.

Other support: National Institutes of Health and the United Cancer Council, Inc.

From the Department of Pediatrics, University of California-San Diego, La Jolla.

THE PROPERTIES OF THE NEUTRAL PROTEINASE RELEASED BY PRIMARY CHONDROCYTE CULTURES AND ITS ACTION ON PROTEOGLYCAN AGGREGATE

The mechanism of proteoglycan catabolism and its regulation is of particular interest, since it has been proposed that increased enzymatic breakdown of proteoglycans may be involved in osteoarthritis. The paper presented here describes the properties of the proteoglycan-degrading enzymes released into the extracellular milieu by chondrocyte cultures that produce cartilage-specific type II collagen but no detectable type I collagen. Since the pH of the extracellular matrix is around neutrality, attention has been focused on enzymes active at neutral pH. Biogel P-60 chromatography of concentrated culture medium showed a major peak of enzyme activity on proteoglycan monomer entrapped in polyacrylamide beads as well as on native proteoglycan aggregates. The enzyme yields a specific limit digestion peptide from the aggregate of approximately 55,000 daltons. The proteolytic enzyme is latent but can be activated by aminophenylmercuric acetate or trypsin. The activity is not inhibited by phenylmethylsulfonyl fluoride or pepstatin but is completely inhibited by *o*-phenanthroline: the activity is restored by Zn or Co ions in the presence of calcium chloride. The release of such metalloproteinase by chondrocytes into the extracellular millieu, its activity at

physiological pH and its ability to degrade native proteoglycans are consistent with a role of the enzyme in proteoglycan metabolism.

Morales, T. I. and *Kuettner, K. E.*

Biochimica et Biophysica Acta 705:92-101, 1982.

Other support: National Arthritis Foundation and the National Institutes of Health.

From the Departments of Orthopedic Surgery and Biochemistry, Rush Medical College, Chicago.

THE RESISTANCE OF EPITHELIA TO VASCULARIZATION: PROTEINASE AND ENDOTHELIAL CELL GROWTH INHIBITORY ACTIVITIES IN URINARY BLADDER EPITHELIUM

The avascular nature of tissues has been studied extensively in a model tissue, hyaline cartilage. Findings indicate that the noted avascularity of epithelia may be attributed to the presence of an extractable, low-molecular-weight factor. This factor contains potent inhibitors of proteolytic enzymes as well as a growth inhibitory activity directed against endothelial cells *in vitro*. The factor is extracted from the epithelium of bovine urinary bladders by 1 M NaCl. The extract is ultrafiltered through an Amicon XM-50 membrane, then concentrated and dialyzed into a 0.9% NaCl solution using a UM-2 membrane. This ultrafiltrate, called the UM-2 retentate (UM-2R), contains approximately 6 μg protein per gram of tissue. The UM-2R has a low content of uronic acid and is practically devoid of hydroxyproline. SDS-PAGE reveals that the UM-2R consists of six major proteins. The UM-2R contains a Trasylol-like proteinase inhibitor that expresses strong trypsin inhibitory activity. Comparisons between bladder and serum UM-2Rs and electrophoretic mobility assays indicate that this proteinase inhibitory activity is derived from the bladder epithelium and not from the serum. The UM-2R is cytotoxic to cultured endothelial cells. Cultures of other cell types (normal and neoplastic) are not affected. It seems, therefore, that the bladder-derived proteinase and endothelial cell growth inhibitory activities may protect epithelia from vascular invasion.

Waxler, B., *Kuettner, K. E.* and *Pauli, B. U.*

Tissue & Cell 14:657-667, 1982.

Other support: National Institutes of Health.

From the Departments of Pathology, Biochemistry and Orthopedic Surgery, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

16. THE REGULATION OF INVASION BY A CARTILAGE-DERIVED ANTI-INVASION FACTOR

This in-depth discussion of the regulation of invasion by a cartilage-derived anti-invasion factor is divided into several major sections including Resistance of normal cartilage to invasion, Susceptibility of extracted cartilage to invasion, and Cartilage-derived anti-invasive factor. A Conclusions section contains the following observa-

tions on the experimental
cartilage to tumor invasion
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anti-invasive activities: (1)
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activity); and (3) tumor gr
molecular-weight (1,000>
hyaline cartilage or, as sho
bovine articular cartilage.
mechanistic pathways by
metastasize to distant sites
motion. Also, they may
growth.

Pauli, B. U. and *Kuettner*

In: Liotta, L. A. and Hart,
Martinus Nijhoff Publishe

Other support: National In

From the Department of Bi
Chicago.

VASCULARITY OF CAR

This book chapter is a
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Kuettner, K. E. and *Pauli,*

In: Hall, B. K. (ed.): *Cartil*

Other support: National In

From the Departments of B
Medical Center, Chicago.

tions on the experimental series which have provided evidence that the resistance of cartilage to tumor invasion may be due to an extractable, low-molecular-weight factor, functionally defined as anti-invasion factor (AIF). This factor contains the following anti-invasive activities: (1) proteinase (collagenase) inhibitory activity; (2) anti-proliferative and anti-migratory activities directed against endothelial cells (anti-angiogenic activity); and (3) tumor growth inhibitory activity. These activities occur in the low-molecular-weight (1,000>MW<50,000) protein fraction of a 1 M NaCl-extract of hyaline cartilage or, as shown only recently, in chondrocyte cultures established from bovine articular cartilage. They may well act as local regulators for some of the major mechanistic pathways by which tumor cells are thought to invade host tissues and metastasize to distant sites, namely by matrix-degrading enzymes and increased locomotion. Also, they may inhibit tumor neovascularization and control local tumor growth.

Pauli, B. U. and *Kuettner, K. E.*

In: Liotta, L. A. and Hart, I. R. (eds.): *Tumor Invasion and Metastasis*, The Hague: Martinus Nijhoff Publishers, 1982, pp. 267-290.

Other support: National Institutes of Health.

From the Department of Biochemistry, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

VASCULARITY OF CARTILAGE

This book chapter is a review of the literature on the special anatomical conditions of the cartilage blood supply and the mechanisms that protect adult cartilage matrix from penetration by vascular endothelium. The perichondrial blood supply, cartilage canals, the growth plate and articular cartilage are all surveyed in this section of the chapter, while mechanisms of the resistance of cartilage matrices to vascular invasion and the resistance of cartilage to tumor cell invasion are discussed fully in other sections. In summary, the conclusions drawn at the end of this paper indicate that the resistance of cartilage to vascular and tumor invasion may be due to an extractable low-molecular-weight factor functionally defined as anti-invasion factor (AIF). This factor can be extracted from uncalcified hyaline cartilage or from cultures of bovine articular chondrocytes by mild salt solutions. The biological activities of the salt extract, such as proteinase inhibition and endothelial cell growth inhibition, are present in a protein fraction that has a molecular weight of between 1,000 and 50,000. These activities may act as local regulators for some of the major mechanistic pathways by which endothelial cells and tumor cells invade host tissues, by matrix-degrading enzymes and by increased proliferation and migration.

Kuettner, K. E. and *Pauli, B. U.*

In: Hall, B. K. (ed.): *Cartilage*, New York: Academic Press, 1982, vol. I, pp. 281-312.

Other support: National Institutes of Health.

From the Departments of Biochemistry and Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

SEPARATION OF A MURINE LEUKEMIA VIRUS PROTEIN KINASE ACTIVITY FROM ITS Pr65^{gag} POLYPROTEIN SUBSTRATE AFTER DNA-CELLULOSE CHROMATOGRAPHY

The murine leukemia virus (MuLV)-associated protein kinase activity has recently been found, *in vitro*, to predominantly phosphorylate Pr65^{gag}, a virus protein present in relatively small amounts in partially purified virus preparations. Other virus proteins, such as p10, Pr27^{gag} and Pr40^{gag}, are also phosphorylated *in vitro*, but to a lesser degree. Furthermore, when immature core subparticles which are enhanced in Pr65^{gag} are prepared from virions by Sepharose 6B exclusion column chromatography, about 50% of the kinase activity (as assayed with the exogenous substrate phosvitin) remains associated with the cores. In this paper, it is reported that this core-associated activity is distinct from Pr65^{gag} since it can be separated from Pr65^{gag} by chromatography on denatured DNA-cellulose columns followed by centrifugation of the 0·2 m-NaCl-eluted fraction. Under these conditions, Pr65^{gag} is pelleted while the kinase activity, which can phosphorylate both endogenous (MuLV Pr65^{gag} and p10) as well as exogenous (phosvitin) substrates, remains in the supernatant. Interestingly, when the amount of Pr65^{gag} is reduced, as in such preparations, p10 then becomes more heavily phosphorylated.

Yoshinaka, Y., Shames, R. and *Luftig, R. B.*

Journal of General Virology 64:95-102, 1983.

Other support: National Cancer Institute and Government of Japan (Y.Y.).

From the Department of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia.

PARAVERTEBRAL MALIGNANT RHABDOID TUMOR IN INFANCY: *IN VITRO* STUDIES OF A FAMILIAL TUMOR

For the study presented here, medical and genetic evaluation was performed on a kindred in which two female siblings died within three months after presenting with paravertebral tumors in the first year of life. The pathology of the two tumors was identical and characteristic of a malignant rhabdoid tumor. There were no identifiable tumor patterns within the kindred which have been associated with any hereditary cancer or precancer syndromes. Fibroblasts were cultured from skin biopsies obtained from the second patient and both parents. Assays of growth kinetics associated with cellular transformation revealed that fibroblasts from the affected sibling can be distinguished from those of the parents and age-matched controls by increased *in vitro* occurrence of tetraploidy. Such evidence suggests that increased *in vitro* tetraploidy occurring spontaneously in cultured fibroblasts is an expression of a cancer-prone gene. Increased *in vitro* tetraploidy has previously been demonstrated in some kindreds with heritable colon cancer syndromes, and it may extend understanding of the genetic etiology of some childhood cancers.

Lynch, H. T. et al.

Cancer 52:290-296, 1983.

Other support: National Cancer Institute, Danes Medical Research Fund, Cornell University Medical College, and the Zemurray Foundation.

From the Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Om
Oncology, the Departme
Pediatric Surgery, Rainb
Cleveland, Case Western
Department of Medicine.

A REVIEW OF HERED
BIOMARKERS IN FAM
SYNDROME

Malignant melanom
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during the past several d
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Lynch, H. T. et al.

Cancer Genetics and Cyt

Other support: National
Order of Eagles.

From the Department of
Dermatology and Boys
Creighton University Sch
Omaha; and the Laborator
York.

GENETICS OF TRANSI
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In this genetic study
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60 mg of *N*-nitroso-*N*-eth
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tumors at approximately f

School of Medicine, Omaha; the Department of Pediatrics, Division of Hematology-Oncology, the Department of Pathology and the Department of Surgery, Division of Pediatric Surgery, Rainbow Babies and Childrens Hospital of University Hospitals of Cleveland, Case Western Reserve University School of Medicine, Cleveland; and the Department of Medicine, Cornell University Medical College, New York.

A REVIEW OF HEREDITARY MALIGNANT MELANOMA INCLUDING BIOMARKERS IN FAMILIAL ATYPICAL MULTIPLE MOLE MELANOMA SYNDROME

Malignant melanoma, which is said to have been described by Hippocrates who was the first to recognize its malignant potential, has shown an increasing incidence during the past several decades. There are several varieties of this ancient disease, sporadic as well as genetic, and in the hereditary varieties of malignant melanoma expression may differ significantly. This in-depth review provides a comprehensive coverage of hereditary malignant melanoma with emphasis upon its heterogeneity as well as newly developed biomarker investigations. The recently described familial atypical multiple mole melanoma (FAMMM) syndrome is featured. Particular attention has been given to findings of increased hyperdiploidy observed as an *in vitro* phenomenon in cultured skin fibroblasts from high-risk and FAMMM-affected subjects. The FAMMM genotype is complex in that it predisposes a patient not only to melanoma (cutaneous and intraocular malignant melanoma) but also to other histologic varieties of cancer, including cancer of the lung, pancreas, and breast. Attention is given to cancer surveillance and management programs for patients at increased risk for the several forms of hereditary malignant melanoma. This approach capitalizes advantageously upon employment of a knowledge of genetics and hereditary cancer syndrome identification, with particular attention to tumor associations.

Lynch, H. T. et al.

Cancer Genetics and Cytogenetics 8:325-358, 1983.

Other support: National Cancer Institute and the Nebraska Division of the Fraternal Order of Eagles.

From the Department of Preventive Medicine and Public Health, the Department of Dermatology and Boys Town Institute for Communication Disorders in Children, Creighton University School of Medicine; University of Nebraska Medical Center, Omaha; and the Laboratory of Cell Biology, Cornell University Medical College, New York.

GENETICS OF TRANSPLACENTALLY INDUCED TERATOGENIC AND CARCINOGENIC EFFECTS IN RABBITS TREATED WITH *N*-NITROSO-*N*-ETHYLUREA

In this genetic study, pregnant rabbits of three partially inbred strains, WH/J, IIIVO/J, and X/J, mated to either IIIVO/J or WH/J males were given ip injections of 60 mg of *N*-nitroso-*N*-ethylurea (NEU) dissolved in trioctanoin on day 18 of gestation. Genetic differences in tumor susceptibility between strains WH/J (low-tumor-incidence strain) and IIIVO/J (high-tumor-incidence strain) have been demonstrated before. Of 22 WH/J × IIIVO/J hybrid progeny weaned, 17 developed primary renal tumors at approximately five months of age. Of 17 IIIVO/J × WH/J hybrid progeny

weaned, 16 developed primary renal tumors at about four months of age. Of five X/J × IIIVO/J hybrid progeny weaned, all developed primary renal tumors at 4.2±0.5 months of age. In addition, eight metastatic nephroblastomas were observed, with the lung being the primary site of metastasis. Neurofibromas were observed in 12 per 22, 12 per 17, and three per five rabbits, respectively, from the various hybrid groups, many of which also had neurolemmal cysts. In addition, one hepatoma and two hemangiomas were observed. Also, teratogenic effects of disproportionate miniaturization, including a left-right reduction in ear length, were observed. Epidermoid cysts also formed and then regressed during the development of these treated animals. On the basis of this study, it appears that susceptibility to the oncogenic effects of NEU appears to have a dominant mode of inheritance, whereas the teratogenic effects of NEU are influenced greatly by the maternal genotype.

Fox, R. R., *Meier, H.*, Bedigian, H. G. and Crary, D. D.

Journal of the National Cancer Institute 69(6):1411-1417, 1982.

Other support: National Cancer Institute, National Science Foundation and the U. S. Public Health Service.

From The Jackson Laboratory, Bar Harbor, ME.

HISTOLOGIC CHARACTERIZATION OF RENAL TUMORS (NEPHROBLASTOMAS) INDUCED TRANSPLACENTALLY IN IIIVO/J AND WH/J RABBITS BY N-ETHYLNITROSOUREA

Following recognition of a genetic predisposition to renal cortical cyst formation and to neoplasms in certain partially inbred strains of laboratory rabbit developed at the Jackson Laboratory, rabbits of the IIIVO/J and WH/J strains were subjected to transplacental administration of N-ethylnitrosourea (ENU). Renal tumors were induced in these strains regardless of whether the carcinogen was administered during the gestation period as a single high systemic dose, as multiple low systemic doses, or as a combination of the precursor amide and nitrite by the oral route. For this study, the histologic features of 63 renal tumors induced in these 39 rabbits were analyzed. All tumors in the series conformed to nephroblastoma, permitting the establishment of histologic standards for this neoplasm in the rabbit as well as observations on tumor progression. Essentially, nephroblastoma proved to be predominantly an epithelial tumor identifying with metanephrogenic blastema, which was presumed to be the tissue of origin during fetal development. The outstanding features comprised clusters or sheets of undifferentiated blastema-like tissue and differentiation along the epithelial pathway into tubular profiles and structures suggestive of primitive, nonvascularized glomeruli. Metastases occurred only in rabbits of strain IIIVO/J, which had been subjected to a single dose of the carcinogen, representing an incidence in this subgroup of 25%. Nephroblastomas resulting from transplacental induction in IIIVO/J rabbits, particularly by single, high doses of ENU, appear to provide a suitable model for the predominant histologic form of the Wilms' tumor complex in man.

Hard, G. C. and Fox, R. R. (*Meier, H.*)

American Journal of Pathology 113:8-18, 1983.

Other support: National Cancer Institute and the National Institutes of Health.

From the Fels Research Institute, Temple University School of Medicine, Philadelphia, and The Jackson Laboratory, Bar Harbor, ME.

SYNTHESIS OF CARTIL IN VITRO. I. ISOLATIO MORPHOLOGY

The studies presented postnatal articular chondro alterations in their phenoty the culture characteristics these studies under a variet cultures. The morphologic isolation and during their s Before culture, chondrocyt first few days of culture the chondrocytes synthesized b were rich in collagen fibrils were most apparent in mas appeared as long streaks an chondrocytes and their matr

Kuettner, K. E., Pauli, B.

The Journal of Cell Biology

Other support: National Ins

From the Departments of B Medical College, Rush-Pre partment of Anatomy, Univ

SYNTHESIS OF CARTILA IN VITRO. II. MAINTENA PHENOTYPE

Collagen-type analysis *in vitro* phenotypic stability sis were performed in roller- gen and proteoglycan, synth course of the culture period. was found predominantly in of Type II and Type III coll medium or the cell-associate and cell-associated matrix ha by cartilage slices or those proteoglycans synthesized by which were readily recovere ciated matrix with low ionic s *vitro* phenotypic stability of were the advantages of this *in* that are capable of effecting a of cartilage matrix.

Kuettner, K. E., Memoli, V. and Daniel, J. C.

SYNTHESIS OF CARTILAGE MATRIX BY MAMMALIAN CHONDROCYTES IN VITRO. I. ISOLATION, CULTURE CHARACTERISTICS, AND MORPHOLOGY

The studies presented here were designed to investigate whether isolated bovine postnatal articular chondrocytes can establish a tissue-like matrix *in vitro*, without alterations in their phenotypic expression. In this paper, the methods of isolation and the culture characteristics of these chondrocytes are outlined. Cells were grown in these studies under a variety of conditions, including a roller-bottle system for mass cultures. The morphologic appearance of these chondrocytes is described during their isolation and during their subsequent elaboration of a cartilage-like matrix *in vitro*. Before culture, chondrocytes were freed of surrounding territorial matrix. Within the first few days of culture they re-established a territorial matrix. As time progressed, chondrocytes synthesized both territorial and extraterritorial matrices. The matrices were rich in collagen fibrils and ruthenium red-positive proteoglycans. These features were most apparent in mass roller cultures in which aggregates of cells and matrix appeared as long streaks and nodules. This morphology revealed an organization of chondrocytes and their matrices similar to that of the parent articular cartilage *in vivo*.

Kuettner, K. E., Pauli, B. U., Gall, G., Memoli, V. A., and Schenk, R. K.

The Journal of Cell Biology 93:743-750, 1982.

Other support: National Institutes of Health.

From the Departments of Biochemistry, Orthopedic Surgery, and Pathology, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, and Department of Anatomy, University of Bern, Bern, Switzerland.

SYNTHESIS OF CARTILAGE MATRIX BY MAMMALIAN CHONDROCYTES IN VITRO. II. MAINTENANCE OF COLLAGEN AND PROTEOGLYCAN PHENOTYPE

Collagen-type analysis was used in this cell culture study to confirm the long-term *in vitro* phenotypic stability of chondrocytes. The results presented for collagen analysis were performed in roller-bottle cultures. The major matrix macromolecules, collagen and proteoglycan, synthesized by the chondrocytes were characterized during the course of the culture period. These cells synthesized mainly Type II collagen, which was found predominantly in the cell-associated matrix. The media contained a mixture of Type II and Type III collagens. Type I collagen was not detectable in either the medium or the cell-associated matrix. The proteoglycan monomers found in the media and cell-associated matrix had the same hydrodynamic sizes as monomers synthesized by cartilage slices or those extracted from adult articular cartilage. The majority of proteoglycans synthesized by the cells were found in high molecular weight aggregates which were readily recovered from the media and were extractable from cell-associated matrix with low ionic strength buffers. The results demonstrate the long-term *in vitro* phenotypic stability of the bovine articular chondrocytes. Also discussed here were the advantages of this *in vitro* system as a model for studying a variety of factors that are capable of effecting alterations in the synthesis, degradation and organization of cartilage matrix.

Kuettner, K. E., Memoli, V. A., *Pauli, B. U.*, Wrobel, N. C., Thonar, E. J.-M. A., and Daniel, J. C.

The Journal of Cell Biology 93:751-757, 1982.

Other support: National Institutes of Health.

From the Departments of Biochemistry, Orthopedic Surgery, and Pathology, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, and the University of Illinois School of Dentistry, Chicago.

CORRELATIONS BETWEEN CELL SURFACE PROTEASE ACTIVITIES AND ABNORMALITIES OF OCCLUDENS JUNCTIONS IN RAT BLADDER CARCINOMA *IN VITRO*

Ultrastructural alterations in zonulae occludentes, which are observed in many malignant tumors, have been examined in considerable detail in urinary bladder carcinomas in humans and laboratory animals. In this attempt to test the hypothesis that proteolytic enzymes play a causative role in abnormalities of zonulae occludentes, the ultrastructure of three carcinoma cell lines which were derived from *N*-[4-(5-nitro-2-furyl)-2-thiozolyl] formamide-induced tumors of the rat urinary bladder were compared *in vitro*. One of these lines had a high cell surface protease activity; the other two lines exhibited relatively low activities. Quantitative electron microscopy data revealed differences in configuration and distribution of zonula occludens-intramembrane fibrils among these cell lines, as indicated by means and standard deviations of zonula occludens widths and numbers of intramembrane fibrils. Although the total length of the intramembranous fibrils per square micrometer of occludens junction area was not statistically different in the three lines, junctional morphology varied greatly. Thus, carcinoma cells with high surface protease activities are able to synthesize near-normal amounts of intramembrane fibrils but are unable to assemble normal zonula occludentes. This indicates that alterations in zonula occludens morphology, which have been induced by exogenous proteolytic enzymes, are identical to those observed in a cell line with high cell surface protease activity.

Pauli, B. U. and Weinstein, R. S.

Cancer Research 42:2289-2297, 1982.

Other support: National Institutes of Health and the Otho S. A. Sprague Memorial Institute.

From the Department of Pathology, Rush Medical College, Rush-Presbyterian-St. Lukes Medical Center, Chicago.

PRODUCTION OF BIOCHEMICAL MARKER SUBSTANCES BY BRONCHOGENIC CARCINOMAS

Growing recognition that neoplastic cells may have several important features that distinguish them from normal cells offers an exciting prospect of more sensitive and highly specific tests for carcinoma—tests that might indicate the presence of a tumor at a time when surgical therapy would offer a greater hope of cure. In the article presented here, data concerning the biochemical products of lung carcinoma cells are reviewed. Close consideration of the material presented shows that pulmonary carcinoma cells produce a spectrum of substances that are not commonly identified in normal cells of the respiratory tract. A large number of these have been evaluated as

potential markers of tumor pre
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from lung tumors does offer s
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duced airway cell injury that pre

Merrill, W. W. and Bondy, P. I

Clinics in Chest Medicine 3(2):2

From the Department of Medicin
CT, and the Pulmonary Section,
Haven, CT.

II. *The*

ENDOGENOUS PROTEIN PHC ACTIVATED HUMAN NEUTR

Numerous types of cells re
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determined by gel electrophoresis
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potential markers of tumor presence. These proteins include tumor-associated hormones, oncofetal antigens, local respiratory tract proteins, and tumor-associated antigens. Although pro-ACTH has been suggested as a sensitive indicator of tumor presence, a more recent report casts some doubt on the sensitivity and specificity of this peptide as a lung cancer marker. Recent research with antigenic substances isolated from lung tumors does offer some hope of a test for cancer detection. Moreover, development of antisera to such antigens offers the possibility of radiolabeling such antibodies as diagnostic and therapeutic tools. Finally, it is possible that alterations in respiratory tract proteins may serve as sensitive early markers of the inhalation-induced airway cell injury that precedes lung cancer.

Merrill, W. W. and Bondy, P. K. *(Reynolds, H. Y.)*

Clinics in Chest Medicine 3(2):307-320, 1982.

From the Department of Medicine, Yale University School of Medicine, New Haven, CT, and the Pulmonary Section, West Haven Veterans Administration Hospital, West Haven, CT.

II. *The Respiratory System*

ENDOGENOUS PROTEIN PHOSPHORYLATION BY RESTING AND ACTIVATED HUMAN NEUTROPHILS

Numerous types of cells respond to external stimuli by altering the levels of phosphorylation of selected proteins. In the study presented here, the possible role of protein phosphorylation in the activation of NADPH oxidase, an enzyme in the plasma membrane of the human neutrophil, was examined in normal and chronic granulomatous disease (CGD) neutrophils by measuring the incorporation of ^{32}Pi into proteins as determined by gel electrophoresis followed by autoradiography. Results showed that resting neutrophils from normal subjects exhibit at least 40 distinct phosphoprotein bands. The level of phosphorylation of these bands was examined after the addition of phorbol myristate acetate (PMA), opsonized zymosan, digitonin, N-formyl-methionyl-leucyl-phenylalanine (FMLP), or NaF. PMA and opsonized zymosan increased the phosphorylation of a set of six protein bands. Digitonin and FMLP consistently caused the phosphorylation of four of these protein bands, while NaF failed to induce increased phosphorylation of any protein band. All activators tested caused the dephosphorylation of one specific protein band. The time relationship between the addition of activating agent, the appearance of the phosphoprotein changes and the onset of NADPH oxidase activity was defined. In another experiment, CGD neutrophils were compared with normal cells for their ability to phosphorylate proteins in response to PMA, and finally, protein phosphorylation was determined in response to NaF, which induces oxidase activation but not degranulation in neutrophils. In summary, the evidence shows that phosphorylation of proteins is a prominent feature of neutrophil metabolism. The striking similarity of phosphorylation changes induced by the various activators tested suggests that protein phosphorylation may play a role in some aspects of neutrophil activation. However, no evidence was obtained regarding a link between protein phosphorylation and activation of NADPH oxidase.

Andrews, P. C. and *Babior, B. M.*

Blood 61(2):333-340, 1983.

Other support: U.S. Public Health Service.

From the Blood Research Laboratory and the Department of Medicine, Tufts-New England Medical Center, Boston.

FATE OF HUMAN NEUTROPHIL ELASTASE FOLLOWING RECEPTOR-MEDIATED ENDOCYTOSIS BY HUMAN ALVEOLAR MACROPHAGES: IMPLICATIONS FOR CONNECTIVE TISSUE INJURY

Although alveolar macrophages (AM) are known to be capable of receptor-mediated endocytosis of neutrophil elastase *in vitro*, little has been known before about the fate of the enzyme after endocytosis. However, in this cell culture study it was seen that after brief exposure to human leukocyte (neutrophil) elastase (HLE) *in vitro*, (1) cell extracts (CEs) of cultured human AM contain detectable quantities of neutrophil elastase and elastase activity for days; (2) endocytosed neutrophil elastase is slowly degraded by the macrophages; (3) neutrophil elastase and elastase activity are slowly released into the culture medium conditioned by the macrophages; (4) elastase activity released into conditioned medium (CM) by the macrophages has catalytic properties of neutrophil elastase; and (5) elastase released into CM during five days in culture is fourfold greater than the initial elastase activity of the CEs, suggesting that enzymatic activity of endocytosed elastase is masked by an intracellular inhibitor. In addition, recent data suggest that binding of HLE by AM triggers release of AM-derived chemotactic factor for neutrophils, which would provide a mechanism for local amplification of inflammatory injury. In summarizing this work, it has been shown that although slowly degraded *in vitro*, HLE endocytosed by AM persists intracellularly for days and is progressively released into the extracellular space with time. These observations could help provide insight into the complex role of mononuclear phagocytes in the modulation of connective tissue injury in disorders such as pulmonary emphysema.

Campbell, E. J. and Wald, M. S.

The Journal of Laboratory and Clinical Medicine 101(4):527-536, 1983.

Other support: U.S. Public Health Service.

From the Pulmonary Division, Department of Medicine, Washington University at The Jewish Hospital of St. Louis, St. Louis.

HUMAN NEUTROPHIL ELASTASE WITHIN HUMAN ALVEOLAR MACROPHAGES: IMPLICATIONS FOR LUNG INJURY

Interest in the involvement of human mononuclear phagocytes in the traffic of "neutrophil" elastase (HLE) in the lung led to this study of the following factors: the fate of HLE after binding to macrophages and the inhibitor of HLE activity found in macrophage extracts. In this work, monolayers of human alveolar macrophages were exposed to 20 μg/ml of purified HLE for 30 minutes and cultured for as long as 4 days. The effect of hypoxia upon the fate of HLE found by macrophages was determined, and extracts of alveolar macrophages were examined for their ability to inhibit HLE. Results of these studies show that: (1) alveolar macrophages slowly degrade endocy-

tosed HLE; (2) enzymatically ac
macrophages; and (3) HLE wi
presence of an endogenous macr
vitro, especially when the cells
complex role in modulating the

Campbell, E. J. and Wald, M.

CHEST 83S:59S-60S, 1983.

Other support: U.S. Public He

From the Pulmonary Disease anc
Washington University at The J

HYPOXIC INJURY TO HUM/ ACCELERATES RELEASE O ELASTASE: IMPLICATIONS INCLUDING PULMONARY E

Earlier studies have shown
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receptor-mediated endocytosis,
within alveolar macrophages fre
gain insight into conditions tha
human neutrophil elastase by alv
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brief exposure to neutrophil elast
bound enzyme, but they also re
amounts; (2) that release of TCA
was enhanced fivefold and twofo
injury during a three-hr. incubati
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Campbell, E. J. and Wald, M. S

American Review of Respiratory

Other support: U. S. Public He

From the Pulmonary Disease and
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tosed HLE; (2) enzymatically active HLE can persist for days within cultured alveolar macrophages; and (3) HLE within alveolar macrophages is largely masked by the presence of an endogenous macrophage inhibitor, but active enzyme can be released *in vitro*, especially when the cells are injured. Thus, macrophages seem to play a very complex role in modulating the "traffic" of the neutrophil elastase.

Campbell, E. J. and Wald, M. S.

CHEST 83S:59S-60S, 1983.

Other support: U.S. Public Health Service.

From the Pulmonary Disease and Respiratory Care Division, Department of Medicine, Washington University at The Jewish Hospital of St. Louis, St. Louis.

HYPOXIC INJURY TO HUMAN ALVEOLAR MACROPHAGES ACCELERATES RELEASE OF PREVIOUSLY BOUND NEUTROPHIL ELASTASE: IMPLICATIONS FOR LUNG CONNECTIVE TISSUE INJURY INCLUDING PULMONARY EMPHYSEMA

Earlier studies have shown that human neutrophil elastase and other neutrophil granulae constituents are internalized by human alveolar macrophages *in vitro* via receptor-mediated endocytosis, and immunoreactive neutrophil elastase is detectable within alveolar macrophages freshly harvested from human smokers. In this attempt to gain insight into conditions that might modulate release of previously internalized human neutrophil elastase by alveolar macrophages, hypoxia was studied as a model of macrophage injury. Results of this study show that: (1) in a three-hr. incubation after brief exposure to neutrophil elastase, control alveolar macrophages partially degraded bound enzyme, but they also released intact, enzymatically active elastase in small amounts; (2) that release of TCA-insoluble radiolabeled elastase and elastase activity was enhanced fivefold and twofold over control, respectively, by alveolar macrophage injury during a three-hr. incubation in humidified nitrogen; (3) that enzymatic activity of bound neutrophil elastase was largely masked by human neutrophil elastase-inhibitory activity of macrophage cell extracts. The data suggest (1) that the fate of neutrophil elastase bound to alveolar macrophages may be modulated by the local tissue environment; (2) that noxious agents may cause proteolytic tissue injury in the vicinity of alveolar macrophages by enhancing release of bound neutrophil elastase; (3) that alveolar macrophages may participate in the pathogenesis of centrilobular pulmonary emphysema by serving as a vector for neutrophil elastase, even if elastase activity is not detectable in alveolar macrophage lysates. In summary, therefore, these results show that alveolar macrophage injury accelerates release into the extracellular space of previously bound human neutrophil elastase, adding to the increasingly complex body of knowledge regarding macrophage modulation of proteinase activity in the extracellular spaces.

Campbell, E. J. and Wald, M. S.

American Review of Respiratory Disease 127:631-635, 1983.

Other support: U. S. Public Health Service.

From the Pulmonary Disease and Respiratory Care Division, Department of Medicine, Washington University at The Jewish Hospital of St. Louis, St. Louis.

MECHANISM OF INHIBITION OF PORCINE ELASTASE BY HUMAN ALPHA-1-ANTITRYPSIN

The interaction of alpha-1-antitrypsin (a plasma glycoprotein which inhibits many proteolytic enzymes) with porcine pancreatic elastase was studied by isolating and characterizing their reaction products. When native alpha-1-antitrypsin and porcine elastase are combined at inhibitor excess, two major products result. One of the products is a complex of the enzyme and inhibitor with amino-terminal ser and val residues, which indicates that a peptide has been removed from the amino-terminal end of the inhibitor. The second product is a modified form of alpha-1-antitrypsin with a mass ratio (Mr) of 51,300, an amino-terminal glx residue and a carboxyl-terminal thr-leu dipeptide. It has no inhibitory activity against elastase. The components of the isolated complex can be split at high pH in the presence of diisopropyl fluorophosphate, which results in a catalytically inactive enzyme with the same Mr and amino-terminal residue as the native enzyme, and a large fragment of alpha-1-antitrypsin. Based on these data, the following hypothesis is proposed by the authors. Elastase can attack alpha-1-antitrypsin at either of two major sites. If it attacks first at the carboxy side of the thr-leu dipeptide, located at the carboxy-terminal portion of the inhibitor, the alpha-1-antitrypsin is cleaved into two fragments with loss of inhibitory activity and absence of complex formation. If, however, the elastase first attacks an x-ser bond near the amino-terminal end of the inhibitor, the elastase then reacts with alpha-1-antitrypsin at the same leu moiety to form a stable complex with complete inhibition of the enzyme.

James, H. L. and *Cohen, A. B.*

Journal of Clinical Investigation 62:1344-1353, 1978.

Other support: U. S. Public Health Service, National Heart, Lung and Blood Institute, and a Pulmonary Program Project.

From the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

THE EFFECTS *IN VIVO* AND *IN VITRO* OF OXIDATIVE DAMAGE TO PURIFIED α_1-ANTITRYPSIN AND TO THE ENZYME-INHIBITING ACTIVITY OF PLASMA

Modification of α_1-antitrypsin by oxidizing agents can be used to study the mechanism of action of α_1-antitrypsin and to develop an animal model of α_1-antitrypsin deficiency. In the study presented here, the kinetics of interaction of purified chloramine T-treated α_1-antitrypsin with porcine pancreatic elastase and trypsin were examined. Oxidized α_1-antitrypsin did not inactivate elastase. However, the oxidized inhibitor could still slowly inactivate trypsin. Oxidation as carried out in these studies converted methionyl residues in α_1-antitrypsin to methionyl sulfoxide. The data indicate that oxidized methionyl residue was more critical to elastase inhibition than to trypsin inhibition and add further weight to the hypothesis that trypsin and elastase bind to two different sites. Also examined were the elastase-inhibiting activities of rabbit, dog and monkey plasma for susceptibility to inactivation by chloramine T. The rabbit has low blood concentrations of elastase-inhibiting activity that is relatively insensitive to oxidative damage. Monkey plasma closely resembles human plasma. Intravenous

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Cohen, A. B.

American Review of Respiratory

Other support: National Heart, L
and a Pulmonary Program Proje

From the Departments of Medic
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ON THE PROPERTIES OF POI COMPLEX WITH HUMAN AL CLEAVAGE

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James, H. L. and *Cohen, A. B.*

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Other support: National Heart, L

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ISOLATION OF SOLUBLE ELA

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infusion of a large dose of chloramine T into a *Macada arctoides* monkey caused a decrease in elastase-inhibiting activity to unmeasurable values, with a concomitant slight increase in immunoreactive α_1-antitrypsin. The animal reverted to full elastase inhibitory activity within 3.7 days. Toxic side effects of hemolytic anemia and methemoglobinemia were minimized by the administration of methylene blue.

Cohen, A. B.

American Review of Respiratory Disease 119:953-960, 1979.

Other support: National Heart, Lung and Blood Institute, U.S. Public Health Service, and a Pulmonary Program Project.

From the Departments of Medicine and Physiology, Temple University Health Sciences Center, Philadelphia.

ON THE PROPERTIES OF PORCINE ELASTASE RELEASED FROM ITS COMPLEX WITH HUMAN ALPHA-1-ANTITRYPSIN BY ALKALINE CLEAVAGE

This study was carried out to investigate the chemical state of the active site serine moiety of the enzyme after alkaline cleavage of the purified alpha-1-antitrypsin-elastase complex. The released elastase component was isolated and compared with the native and diisopropyl fluorophosphate (DFP)-inhibited enzyme. Results showed that most of the elastase component precipitated on returning the pH to neutral. The elastase component and the native enzyme subjected to the same conditions of pH and temperature reacted to approximately the same extent with radioactively labeled DFP. There were about two moles of dehydroalanine per mole of enzyme either in the presence or absence of complex formation. Thus, the enzyme is still capable of reacting with DFP or it is denatured and inactivated by partial conversion of cystine residues to dehydroalanine. Anhydroelastase is apparently not formed during cleavage of the complex.

James, H. L. and *Cohen, A. B.*

Biochemical and Biophysical Research Communications 90(2):547-553, 1979.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

ISOLATION OF SOLUBLE ELASTIN-LATHYRISM

Since studies aimed at examining elastin structure and biosynthesis necessitate sufficient amounts of purified and chemically characterized tropoelastin to use as a standard, an efficient and relatively inexpensive method for obtaining tropoelastin is clearly desirable. This report describes the development and use of experimentally induced lathyrism as a means of obtaining tropoelastin from a variety of different elastic tissues. Lysyl oxidase is the enzyme responsible for catalyzing the oxidative deamination of lysyl residues, which is a key step in the insolubilization of elastin via the formation of cross-links, such as desmosine and isodesmosine. Lysyl oxidase can be inhibited by rendering animals copper-deficient, since lysyl oxidase requires Cu^{2+}

for activity. Since lathyrogens are also known to inhibit lysyl oxidase, an alternative to copper deficiency is the administration of a lathyrogen directly to the normal diet of an animal. The methodology described in a section on the induction of lathyrism in chickens represents optimum conditions for isolating tropoelastin from chick tissues. In this study the chicks generally showed definite symptoms of lathyrism (the most obvious being the inability to move about the cages) within 5-8 days after initiation of the lathyrogen feeding. Also, piglets just weaned showed signs of lathyrism after 9 days on the lathyrogen diet. The isolation of tropoelastin is described in this paper. This procedure employed on chick aortic tissue results in a homogeneous protein of 70,000 daltons, which corresponds to the tropoelastin b species of soluble elastin. Several other procedures described here are all designed for the isolation of tropoelastin b, the 70,000 dalton species. To obtain any tropoelastin a, the 73,000 dalton species of soluble elastin, other steps must be taken also, since a simple preparative procedure for purifying significant amounts of tropoelastin a has not yet been developed.

Rich, C. B. and *Foster, J. A.*

Methods in Enzymology 82:665-673, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

CHARACTERIZATION OF TRACHEAL MAST CELL REACTIONS IN VIVO: INHIBITION BY A BETA-ADRENERGIC AGONIST

The purpose of the present study was to test the suitability of a new experimental preparation, the isolated tracheal segment, for examining the relationship between endogenous beta-adrenergic stimulation by sympathetic nerves, or endogenous cholinergic stimulation by parasympathetic nerves, and the severity of mast cell reactions in the airways. To do this, the effects of pharmacologic beta-adrenergic stimulation on the severity of tracheal mast cell reactions were tested in 12 anesthetized dags *in vivo*. In control dogs, tracheal arterial injections of compound 48/80 produced arteriovenous differences in histamine concentrations across the trachea, but not the lung; this finding suggested selective degranulation of tracheal mast cells. Tracheal arteriovenous histamine differences correlated with tracheal contractions induced by compound 48/80 in these animals. In other dogs, injections of isoproterenol before compound 48/80 prevented the development of both tracheal arteriovenous differences and contractions. Since it was seen here that pharmacologic beta-adrenergic stimulation inhibited degranulation of tracheal mast cells, it appears that the tested preparation is suitable for examining the effects of endogenous stimuli on the severity of mast cell reactions in the central airways of anesthetized dogs.

Brown, J. K., Leff, A. R., Frey, M. J., Reed, B. R., Lazarus, S. C., Shields, R., and *Gold, W. M.*

American Review of Respiratory Disease 126:842-848, 1982.

Other support: U. S. Public Health Service.

From the Cardiovascular Research Institute and the Department of Medicine, University of California, San Francisco.

BIOCHEMICAL AND PHYSI
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Leff, A. R., Brown, J. K., Fre

Journal of Applied Physiology:
1983.

Other support: National Heart,
and the Chicago Community Tr

From the Cardiovascular Resea
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AUGMENTATION OF α-ADR
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BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF COMPOUND 48/80 ON CANINE TRACHEA *IN VIVO*

This study was designed to examine both the biochemical and physiological mechanisms regulating the release of histamine (from mast cells) by compound 48/80 in a central airway of living dogs. To do this, compound 48/80 was injected selectively into the tracheal circulation and the effects on histamine content in the tracheal posterior membrane and on isometric tracheal smooth muscle tension were examined. Results showed that in four dogs compound 48/80 caused an increase in tracheal tension [13± 5 (SD) g/cm], while femoral arterial blood pressure decreased only 14 ±11%. Tracheal tissue histamine decreased 17 ± 6% in five dogs receiving intra-arterial compound 48/80. Chlorpheniramine, an H_1-antagonist, selectively inhibited tracheal contraction to compound 48/80 and histamine. Cimetidine, an H_2-antagonist, did not alter the response to intra-arterial histamine. In 11 dogs, the doses of both intraarterial histamine and acetylcholine required to produce a threshold increase in tracheal tension of 8 g/cm were compared. Threshold doses for acetylcholine varied 10-fold, compared with 100-fold variation for histamine among these dogs. There was a significant correlation between increased tracheal tension produced by compound 48/80 and histamine. Based on this data, it was concluded that compound 48/80 causes a variable increase in tracheal tension *in vivo* because of marked variability in the H_1-receptor response of tracheal smooth muscle to histamine and because of variability in the release of mediator from respiratory mast cells by compound 48/80.

Leff, A. R., Brown, J. K., Frey, M., Reed, B., and *Gold, W. M.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(3):720-729, 1983.

Other support: National Heart, Lung and Blood Institute, Chicago Lung Association, and the Chicago Community Trust.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, and the Pulmonary Section, Pritzker School of Medicine, University of Chicago, Chicago.

AUGMENTATION OF α-ADRENERGIC CONTRACTIONS IN THE TRACHEALIS MUSCLE OF LIVING DOGS

The major goal of this study was to test for augmentation of α-adrenergic responsiveness by histamine in the trachealis muscle of dogs. To do this, an *in situ* preparation was used to test the effects of histamine and other agents on α-adrenergic responses in the trachealis muscle of 34 anesthetized dogs. Under basal conditions, maximum α-adrenergic responses were small compared with those induced by a supramaximal parasympathetic stimulus. After exposure of the muscle to histamine, responsiveness increased markedly to α-adrenergic stimuli, which included tracheal arterial injections of norepinephrine and phenylephrine or electrical stimulation of sympathetic nerves. Augmented α-adrenergic responsiveness persisted for 20 minutes after the end of contractions induced by histamine. Serotonin and long-acting, but not short-acting, cholinergic agonists also potentiated α-adrenergic responsiveness. From these observations, it was concluded that exposure of the trachealis muscle in living dogs to a variety of constrictor stimuli potently augmented its α-adrenergic responsiveness.

Brown, J. K., Shields, R., Jones, C. and *Gold, W. M.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(6):1558-1566, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, and the Respiratory Care Section, Department of Medicine, Veterans Administration Medical Center, San Francisco.

TRIACYLGLYCEROL HYDROLYSIS IN THE ISOLATED, PERFUSED RAT LUNG

The mechanism of hydrolysis of circulating triacylglycerols was investigated in the isolated, perfused rat lung. For this study, hydrolysis of emulsified tri[^{3}H]oleate or doubly labeled [^{3}H]glyceryl, tri[^{14}C]oleate was measured by quantitation of [^{3}H]oleate or of [^{14}C]oleate and [^{3}H]glycerol released into the perfusate. Results show that triacylglycerols are hydrolyzed during perfusion through the isolated rat lung. The release of free fatty acids was linear after an initial lag period, the length of which was inversely proportional to the triacylglycerol concentration. Studies with doubly labeled [^{3}H]glyceryl, tri[^{14}C]oleate showed that, during triacylglycerol hydrolysis, the molar ratio of free fatty acid to glycerol released is close to 1, suggesting that about one-third of the fatty acids hydrolyzed is released into the pulmonary circulation. The earlier appearance of free fatty acid than glycerol in the venous effluent indicates that the first step in triacylglycerol hydrolysis occurs at the endothelial surface. Heparin was administered to investigate the role of lipoprotein lipase in this process. Heparin administered 10 minutes prior to perfusion led to marked release of lipoprotein lipase from the lungs and completely abolished the subsequent hydrolysis of circulating triacylglycerols. Perfusions carried out four hours after heparin administration show that endothelial lipoprotein lipase levels do not return to normal within four hours in the lung. In summary, the data show that circulating triacylglycerols are hydrolyzed by endothelial lipoprotein lipase during passage through the lung.

Gal, S., Bassett, D. J. P., *Hamosh, M.*, and Hamosh, P.

Biochimica et Biophysica Acta 713:222-229, 1982.

Other support: National Institutes of Health.

From the Departments of Physiology and Biophysics, and of Pediatrics, Georgetown University Medical Center, Washington, DC.

HYDROLYSIS OF TRIGLYCERIDES IN THE ISOLATED PERFUSED RAT LUNG

Dietary fatty acids are transported in the plasma as large size triglycerides which cannot cross the endothelial barrier until they are hydrolyzed. The study presented here was undertaken to determine the hydrolysis of different saturated and unsaturated triglyceride-fatty acids into the tissue lipids of the isolated perfused rat lung. Results showed that saturated triglycerides were hydrolyzed at significantly lower rates than unsaturated triglycerides; tricaprylin, trimyristin and tripalmitin were hydrolyzed

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Compton, S. K., *Hamosh, M.*

Lipids 17(10):696-702, 1982.

Other support: National Inst

From the Departments of Phy
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Yamada, K. A., Norman, W.

Brain Research 248:71-78, 19

Other support: American Hea
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at 8.1 + 1.8, 5.4 + 1.5 and 9.5 + 1.8 μmol free fatty acids/g dry wt/100 min, respectively, whereas triolein and trilinolein were hydrolyzed at 20.2 + 1.8 and 20.6 + 0.3 μmol free fatty acids/g dry wt/100 min, respectively. The polyunsaturated triglycerides, trilinolein and triarachidonin were hydrolyzed at even higher rates. Labeled unsaturated triglycerides were broken down at significantly higher rates than labeled saturated triglycerides. Also, it was seen that incorporation of triglyceride-fatty acid into lung lipid was greater into neutral lipids than into phospholipids. In conclusion, two factors that appear to affect lung lipoprotein lipase activity are composition of circulating triglyceride-fatty acids and concentration of circulating triglycerides. Uptake and incorporation of fatty acids into tissue lipids were shown here to be proportional to the rate of hydrolysis of the particular substrate. It seems, therefore, that the triglyceride-fatty acids can be used by the lung for metabolic needs.

Compton, S. K., *Hamosh, M.* and Hamosh, P.

Lipids 17(10):696-702, 1982.

Other support: National Institutes of Health.

From the Departments of Physiology and Biophysics, and of Pediatrics, Georgetown University Medical Center, Washington, DC.

MEDULLARY-VENTRAL SURFACE GABA RECEPTORS AFFECT RESPIRATORY AND CARDIOVASCULAR FUNCTION

This study deals with the effect of γ-aminobutyric acid (GABA), muscimol, and bicuculline on respiration, arterial blood pressure and heart rate after direct application to the ventral surface of the medulla. In order to localize the site of action, Perspex rings were placed directly onto the brainstem surface over one of three areas designated as Schlaefke's, Mitchell's or Loeschcke's. Application of muscimol (0.25-2.66 μg) to Schlaefke's area in six cats reduced minute ventilation from 443 ± 38 to 291 ± 52 ml/min by reducing tidal volume from 31.8 ± 2.3 to 17.6 ± 1.4 ml, without changing respiratory rate and duration of inspiration. Hypotension and bradycardia were also observed. Application of GABA (0.14-4.86 mg) produced similar effects on respiratory activity and arterial blood pressure. No significant effects occurred when high doses of these agents were applied to Loeschcke's and Mitchell's areas. Application of bicuculline (5-25 μg) to Schlaefke's area had the opposite effect of muscimol and GABA on respiratory activity and blood pressure, and reversed the respiratory and cardiovascular depressant effects of both agents. On the basis of this study, the authors concluded that GABA receptors are present at Schlaefke's area, and that activation of these receptors results in respiratory depression, hypotension, and bradycardia. Moreover, these results suggest that GABA may be an important inhibitory neurotransmitter in the modulation of respiratory and cardiovascular control.

Yamada, K. A., Norman, W. P., *Hamosh, P.*, and Gillis, R. A.

Brain Research 248:71-78, 1982.

Other support: American Heart Association and the Lilly Research Laboratories, Eli Lilly.

From the Departments of Pharmacology, Anatomy, and Physiology, Georgetown University Schools of Medicine and Dentistry, Washington, DC.

CIGARETTE SMOKE BLOCKS CROSS-LINKING OF ELASTIN *IN VITRO*

The three-dimensional structure of elastin is highly stabilized by covalent cross-links between adjacent elastin peptide chains. Desmosine and isodesmosine are the major cross-linking amino acids in elastin and are synthesized by condensation of three residues of α-aminoadipic-δ-semialdehyde (lysinal) with one lysine residue. In the study reported here, it is shown that water-soluble components of the gas phase of filtered cigarette smoke inhibit the formation of covalent desmosine cross-links during conversion of tropoelastin to elastin *in vitro*. These same components also suppress lysyl-oxidase-catalyzed oxidation of lysine ϵ-amino groups in tropoelastin (the chemical step preceding formation of all elastin cross-links, including desmosine) in a dose-dependent fashion. However, gas phase smoke does not block the oxidation of diaminopentane by lysyl oxidase. Thus, gas phase cigarette smoke may possess substrate-directed (rather than enzyme-directed) inhibitory components capable of interfering with elastin cross-linking *in vitro*.

Laurent, P., *Janoff, A.* and Kagan, H. M.

American Review of Respiratory Disease 127(2):189-192, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook, and the Department of Biochemistry, Boston University School of Medicine, Boston.

HUMAN METHIONINE SULFOXIDE-PEPTIDE REDUCTASE, AN ENZYME CAPABLE OF REACTIVATING OXIDIZED ALPHA-1-PROTEINASE INHIBITOR *IN VITRO*

Previous studies have shown that *Escherichia coli* contains an enzyme, methionine sulfoxide [Met(0)]-peptide reductase, capable of catalyzing the reduction of Met(0) residues in proteins and it has also been shown that this enzyme can reactivate chemically oxidized alpha-1-proteinase inhibitor (α_1PI) *in vitro*. The study presented here demonstrates the presence of Met(0)-peptide reductase activity in human lung homogenates and in lysates of polymorphonuclear leukocytes (PMN) and alveolar type II cells. Enzyme activity was not detected in human bronchoalveolar lavage fluid or in pulmonary alveolar macrophage lysates. The Met(0)-peptide reductase derived from PMN is capable of reactivating α_1PI oxidized by treatment with chloramine-T or a myeloperoxidase oxidizing system. However, the PMN-derived enzyme does not reactivate α_1PI inactivated by treatment *in vitro* with aqueous solutions of cigarette smoke plus peroxide. Also, after instillation of oxidized human α_1PI into lungs of normal or ozone-treated rats, no reactivated α_1PI could be found in the pulmonary lavage obtained from these animals. Finally, patients with chronic obstructive pulmonary disease appear to have normal levels of PMN Met(0)-peptide reductase.

Carp. H., *Janoff, A.*, Abrams, W., *Weinbaum, G.*, Drew, R. T., Weissbach, H., and Brot, N.

American Review of Respiratory Disease 127(3):301-305, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook; the

Research Section, Departmen
phia; the Medical Departmen
Roche Institute of Molecular

PROTEASES AND LUNG I

This minireview of ongo
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Janoff, A.

CHEST 83(5):54S-58S, 1983.

Other support: U. S. Public H

From the Department of Pathc

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Research Section, Department of Medicine, Albert Einstein Medical Center, Philadelphia; the Medical Department, Brookhaven National Laboratory, Upton, NY, and the Roche Institute of Molecular Biology, Nutley, NJ.

PROTEASES AND LUNG INJURY: A STATE-OF-THE-ART MINIREVIEW

This minireview of ongoing knowledge about the relationship between proteases and lung injury is presented in the following sections: Historical Perspectives; Highlights of Recent Developments (1. Mononuclear Phagocytes; Role in Elastase Homeostasis, 2. Oxidative Inactivation of Elastase-inhibitors, and 3. Immunochemical Assays of Elastin Degradation); Current Major Questions; and Some Suggestions for Future Study. In summary, it is widely supposed today that the destructive changes in lung parenchyma associated with pulmonary emphysema are mediated in large part by unrestrained proteolytic activity in lung connective tissue. This condition is thought to arise whenever elastolytic proteases are released from lung cells and are ineffectively downregulated by endogenous proteinase inhibitors in the lower respiratory tract. Marginated neutrophils, resident alveolar macrophages, and (more recently) freshly recruited mononuclear leukocytes in transit between blood capillaries and alveolar airspaces have been implicated as likely sources of lung-damaging elastases. On the other hand, alpha$_1$-proteinase inhibitor (α_1Pi) has been implicated as an important regulator of neutrophil and monocyte elastases. The hypothesis that an imbalance between these factors, favoring protease activity, is pathogenetic in emphysema rests largely on the signal observations of Laurell and Eriksson who recognized that selected cases of early-onset familial emphysema were associated with heritable α_1-Pi deficiency, and Gross *et al.* who showed that intrapulmonary instillation of elastolytic proteases produced anatomic derangements in experimental animals characteristic of human emphysema. Recent progress in this area has been exciting and has been the subject of discussion at several major symposia over the last decade. However, protease-antiprotease imbalance as the mechanism of alveolar effacement in most forms of emphysema still remains to be proven.

Janoff, A.

CHEST 83(5):54S-58S, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook.

CIGARETTE SMOKE BLOCKS CROSS-LINKING OF ELASTIN *IN VITRO*

For the assay system reported here, purified tropoelastin and lysyl oxidase were incubated for 24 hrs. to facilitate tropoelastin coacervation and the resultant desmosine was measured by a previously described radioimmunoassay method. Three different kinds of cigarette smoke solution were tested in this assay system. Results of the five experiments described here showed that no desmosine was detected under baseline conditions. However, using complete enzyme-substrate mixtures and incubating at 37° C in the absence of smoke, desmosine formation was demonstrated both with homologous enzyme-substrate mixtures and heterologous mixtures. On the other hand, in the presence of aqueous smoke solutions, desmosine synthesis was inhibited in all five experiments. When an independent assay method was used, an inhibitory effect of gas

phase cigarette smoke on the chemical reaction which precedes and is required for desmosine formation was also shown. In these experiments, lysyl oxidase catalyzed oxidative deamination of lysine ϵ-amino groups was monitored. The effect of gas phase smoke on elastin oxidation was dose-dependent, and inhibition was readily detectable even at dilutions of smoke solution as high as 1:150. Oxidation of lysine ϵ-amino groups precedes synthesis of all known elastin cross-links. Thus, the data presented here support the conclusion that cigarette smoke can inhibit formation of other elastin crosslinks besides desmosine.

Laurent, P., *Janoff, A.* and Kagan, H. M.

CHEST 83(5):63S-65S, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook, and the Department of Biochemistry, Boston University School of Medicine, Boston.

LEVELS OF ELASTASE ACTIVITY IN BRONCHOALVEOLAR LAVAGE FLUIDS OF HEALTHY SMOKERS AND NONSMOKERS

The purpose of this study was to compare elastase levels in lung washes of healthy volunteer smokers and nonsmokers and to partly characterize any enzymes found with respect to their chemical classification and cellular source(s) within the lung. In order to do this, elastase activity was measured in concentrated, cell-free bronchoalveolar lavage (BAL), using the synthetic substrate butyloxycarbonyl-L-alanyl-L-alanyl-L-prolyl-L-valyl-amino-methylcoumarin. The BAL fluids obtained from young, asymptomatic smokers with normal urine desmosine concentrations one hour after they had smoked two cigarettes showed significant increases in elastase levels compared with those in nonsmoking control subjects. Repeated BAL samples were obtained at later times from one smoker with a high initial enzyme value and from one nonsmoking control subject. Elastase activity varied over time, but both subjects consistently remained within their respective group ranges. Inhibition studies on pooled BAL from smokers showed that the elastase activity present had properties of both serine and metalloenzymes, suggesting that neutrophils and/or monocytes (serine enzyme) as well as macrophages (metalloenzyme) contributed to the observed activity. Lung lavage cells obtained from two of the smokers and two of the nonsmokers were stained with both a chromogenic substrate and by indirect immunofluorescence for the serine enzyme. The results of these and other studies show some asymptomatic smokers have significantly more elastase activity in their bronchopulmonary secretions than do nonsmokers (as measured with a low molecular weight synthetic substrate). Also, the enzyme activity recovered in smokers' BAL appears to be derived mainly from neutrophils (serine enzyme) and macrophages (metalloenzyme), rather than from monocytes.

Janoff, A., Raju, L. and Dearing, R.

American Review of Respiratory Disease 127(5):540-544, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York at Stony Brook, and the Pulmonary Disease Section, Nassau County Medical Center, East Meadow, NY.

THE ROLE OF OXIDATIVI

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Janoff, A. et al.

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Other support: U. S. Public H

From the Department of Patho

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Janoff, A.

Journal of Applied Physiology:
1983.

Other support: National Heart.

From the Department of Patho
Stony Brook.

THE ROLE OF OXIDATIVE PROCESSES IN EMPHYSEMA

Both proteases and activated species of oxygen have been implicated as mediators of immunologic lung injury, and the present report attempts to link these two pathways of connective tissue destruction. For instance, elastase/elastase inhibitor imbalance in the lung has been implicated in the pathogenesis of pulmonary emphysema. In light of this, it may be significant that the activity of two major elastase inhibitors, alpha 1-proteinase inhibitor (alpha 1-antitrypsin, α_1PI) and bronchial mucus proteinase inhibitor, can be decreased by oxidizing agents. The effect can be observed with ozone, substances present in cigarette smoke, and oxygen metabolites generated by lung macrophages as well as peroxidative systems released by other phagocytic cells. Thus, α_1PI recovered from lung washings of cigarette smokers has only half the predicted normal activity per mg inhibitor and contains four moles of methionine sulfoxide (oxidized methionine) per mole of inactive inhibitor. By contrast, α_1PI purified from nonsmokers' lung washings is fully active and contains only native methionine. At the same time, lung washes from some smokers show significantly greater hydrolytic activity against a specific synthetic elastase substrate than do lung washes of nonsmokers. In addition to the possible effect of cigarette smoking on lung elastase/elastase inhibitor balance, it seems smoking also may interfere with elastin repair mechanisms. Specifically, it has been seen that acidic water-soluble gas phase components of cigarette smoke prevent synthesis of desmosine cross-links during elastinogenesis *in vitro*.

Janoff, A. et al.

American Review of Respiratory Disease 127(2):S31-S38, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook.

BIOCHEMICAL LINKS BETWEEN CIGARETTE SMOKING AND PULMONARY EMPHYSEMA

It is widely believed today that the destructive changes associated with emphysema are mediated in large part by unrestrained proteolytic (especially elastolytic) activity in lung connective tissue. It is further argued that this last condition results from the heightened release of elastolytic proteases by cells in the lung, coupled with an acquired or genetic deficiency in the endogenous antiproteases responsible for regulating these enzymes. Even though this hypothesis still requires rigorous proof, newer information is rapidly developing that links the protease-pathogenesis model to an environmental risk factor associated with disease, namely cigarette smoking. A triad of chemical and cellular effects produced by cigarette smoke has been suggested as contributing to altered elastin metabolism and eventual development of emphysema in smokers. The present article reviews some of these observations, seeks to place them within the overall framework of the protease model and attempts to raise questions for future study.

Janoff, A.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 55(2):285-293, 1983.

Other support: National Heart, Lung and Blood Institute.

From the Department of Pathology, State University of New York at Stony Brook, Stony Brook.

PROTEASES AND ANTIPROTEASES IN THE LUNG

Proteases and antiproteases in the lung have received increasing attention in the past 15 years because of growing evidence that an imbalance between proteolytic enzymes and their inhibitors may give rise to certain features of chronic obstructive lung diseases (COLD) in man. For example, unrestrained elastolytic activity in the lung has been suggested to be responsible for the alveolar deformation seen in pulmonary emphysema, while several workers have shown that elastolytic attack also causes alterations in the conducting airways similar to those associated with chronic bronchitis. The first section of this paper is devoted to proteases in the lung (proteases of the alveolar macrophage and of the polymorphonuclear neutrophil (PMN)) and to potential targets of macrophage and PMN elastases in lung connective tissue. Following sections consider antiproteases in the lung, elevation of lung proteases in smokers, depression of lung antiproteases in smokers, inactivation of antiproteases by oxidants in tobacco smoke, and inactivation of antiproteases by cell-generated oxidants. Summation of this detailed review chapter shows that some of the elastolytic proteases in the lung and some of the major lung antiproteases that regulate the activity of these enzymes are discussed. The investigators then propose potential mechanisms by which cigarette smoking could elevate the elastase "burden" of the lung and/or depress the lung's elastase-inhibitor "screen." Finally, the protease-pathogenesis model of COLD is reexamined in an attempt to show how cigarette smoking and other environmental and genetic factors might influence the balance between proteases and antiproteases in the lung.

Janoff, A. and Carp, H.

In: Newball, H. H. (ed.): *Immunopharmacology of the Lung*, New York:Marcel Dekker, Inc., 1983, pp. 173-208.

Other support: National Heart, Lung and Blood Institute.

From the Department of Pathology, State University of New York, Stony Brook.

CROSS-CIRCULATION STUDIES ON THE INFLUENCE OF HYPOXIA AND HYPOXEMIA OF NEUROEPITHELIAL BODIES IN YOUNG RABBITS

Using young rabbits for testing, the reactions of neuroepithelial bodies (NEB) to (1) hypoxia with normoxemia in the arteria pulmonalis on the one hand, and (2) hypoxemia in the arteria pulmonalis with normoxic aeration on the other hand, were investigated by means of cross-circulation experiments and light microscopic, electron microscopic and morphometric techniques. Results showed that hypoxically aerated young rabbits, which received normoxemic blood in their arteria pulmonalis from a donor rabbit by means of an arterio-arterial cross-circulation with mutual exchange transfusion, revealed an increased exocytosis of the dense-core vesicles of their NEB. Normoxically aerated young rabbits which received hypoxemic blood in an identical manner, did not exhibit an increased exocytosis. In light of these results, it is concluded that the NEB apparently react directly to the hypoxia of the inhaled air and not to the hypoxemia of the pulmonary blood. By the release of serotonin and a polypeptide substance, they may produce a local vasoconstriction in hypoxically aerated lung areas, enabling an intrapulmonary regulation of the V/Q ratio. This is regarded as additional proof that the NEB, while being modulated by the CNS, probably are

intrapulmonary chemoreceptors
sition of the inhaled air.

Lauweryns, J. M. et al.

Cell and Tissue Research 193:3

Other support: Nationaal Fond

From the Laboratory of Histopa
Medicine, Leuven, Belgium.

INTERACTION OF SEROTOI
BRONCHOCONSTRICTION I

In this attempt to determi
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Sheller, J. R., Holtzman, M. J.

Journal of Applied Physiology: ,
1982.

Other support: National Institut

From the Cardiovascular Researc
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intrapulmonary chemoreceptors with local secretory activities, reacting to the composition of the inhaled air.

Lauweryns, J. M. et al.

Cell and Tissue Research 193:373-386, 1978.

Other support: Nationaal Fonds voor Wetenschappelijk Onderzoek, Belgium.

From the Laboratory of Histopathology, Katholieke Universiteit te Leuven School of Medicine, Leuven, Belgium.

INTERACTION OF SEROTONIN WITH VAGAL- AND ACh-INDUCED BRONCHOCONSTRICTION IN CANINE LUNGS

In this attempt to determine whether serotonin acts at the level of the airway smooth muscle muscarinic receptor, a comparison was made of the effect of serotonin on bronchoconstriction caused by vagal stimulation and by inhalation of acetylcholine (ACh) aerosols. To do this, the bronchoconstrictor response to electrical stimulation of the peripheral ends of both cut cervical vagus nerves was potentiated by serotonin aerosols in 10 experiments in seven anesthetized dogs. The bronchoconstrictor response to acetylcholine aerosols was unchanged after serotonin. Specifically, the bronchoconstriction caused by electrical stimulation of vagal nerves was significantly increased by pretreatment with serotonin, but the response to inhaled ACh was unchanged. The potentiation of the effect of vagal stimulation did not result from alteration in base-line airway caliber, because base-line R_L was not changed by serotonin aerosols. Because of these results, it was concluded that the smooth muscle muscarinic receptor is not the site where serotonin interacts with the vagal motor pathway to airway smooth muscle. It seems, rather, that serotonin acts at the level of the parasympathetic ganglia or the postganglionic nerve terminal to potentiate the bronchoconstrictor response to vagal stimulation.

Sheller, J. R., Holtzman, M. J., Skoogh, B-E., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52(4):964-966, 1982.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

REFLEX STIMULATION OF TRACHEAL MUCUS GLAND SECRETION BY GASTRIC IRRITATION IN CATS

This study had two aims: (1) to determine whether flow from the submucosal glands of the trachea is reflexly regulated by sensory stimuli from the stomach in the cat, and (2) if such a gastropulmonary reflex exists, what sensory and motor pathways are important. Responses tested here included mechanical stimulation, blockade of the secretory response and atropine sulfate infusion followed by electrical stimulation of the central end of either vagus nerve. Results showed that mechanical stimulation of the gastric mucosa increased submucosal gland secretions from 7.9±0.7 to 17.4±1.7

nl/min (mean±SE, $P<0.001$). This effect was prevented reversibly by cooling both abdominal vagus nerves to −3° C before stimulation and was restored by rewarming the nerves. The effect was prevented irreversibly by cutting both abdominal vagus nerves and was then mimicked by electrically stimulating the central cut end of one of the nerves. This increase in secretions caused by electrical stimulation of the nerve was prevented by administration of atropine sulfate before stimulation. It was concluded, therefore, that stimuli from the stomach reflexly affect the rate of submucosal gland secretion. The sensory limb of this reflex lies in the abdominal vagus nerves, and the motor pathways are mediated by cholinergic muscarinic receptors.

German, V. F., Corrales, R., Ueki, I. F., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52(5):1153-1155, 1982.

Other support: National Heart, Lung, and Blood Institute and Vick Divisions Research and Development.

From the Cardiovascular Research Institute and Departments of Medicine, Pediatrics and Physiology, University of California, San Francisco.

SELECTIVE EFFECT OF GENERAL ANESTHETICS ON REFLEX BRONCHOCONSTRICTOR RESPONSES IN DOGS

Although anesthesia may be critical in the study of bronchoconstrictor responses and, in particular, the study of reflex responses, the sites of action of general anesthetics in the reflex pathway are unclear. Therefore, in this attempt to determine which part of the parasympathetic bronchoconstrictor pathway is most sensitive to depression by general anesthetics, the authors stimulated different parts of the pathway in dogs after initial anesthesia with chloralose and urethan and then after additional anesthetic drugs. The entire reflex pathway was stimulated by producing apnea or hypoventilation, the sensory pathway by electrically stimulating the proximal ends of cut superior laryngeal nerves, and the motor pathway by stimulating the distal end of a cut cervical vagus nerve. Bronchoconstrictor responses to all stimuli were assessed with a bypassed tracheal segment. When no additional anesthetic was administered, responses to all stimuli increased with time. Small additional doses of anesthetics (thiopental, 1-5 mg/kg; pentobarbital, 1-2 mg/kg; amobarbital, 1-2 mg/kg; or chloralose, 10 mg/kg) decreased responses to reflex and sensory stimulation markedly and reversibly, but they did not affect responses to motor stimulation. Increased doses decreased responses to motor stimulation as well. While a previous study showed that barbiturates depress parasympathetic ganglionic synapses, this study suggests that central nervous system synapses may be even more sensitive to depression by general anesthetics.

Holtzman, M. J., Hahn, H. L., Sasaki, K., Skoogh, B.-E., Graf, P. D., Fabbri, L. M., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(1):126-133, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

DOSE-DEPENDENT INHIBI BRONCHOCONSTRICTION

Inhalation of cold air is a w asthma. Observations like this dealing with bronchoconstrictic treatment with atropine or antim taken to determine whether inh choconstriction in a dose-depen assessed the effects of placebo a on base-line specific airway resis 5 min of voluntary eucapnic hyp assessed the effect of the lowest five breaths of 1.0% methacho maximal reduction in base-line methacholine on sRaw, but it d Higher doses of atropine did inh fashion. The dose of atropine re increase in sRaw produced by cc causes bronchoconstriction thro carinic agents may be required those required to reduce base-lin an inhaled muscarinic agonist.

Sheppard, D., Epstein, J., Holt

Journal of Applied Physiol: Resp

Other support: National Heart. sources Board.

From the Cardiovascular Researc ogy, University of California, S General Hospital, San Francisc

BARBITURATES DEPRESS V TRACHEA AT GANGLIA

Barbiturates have been sho stimulation of the vagus nerve. vagal motor pathway to airway barbiturates, isometric muscle t pathway at four different sites bet In isolated tracheal rings from nction were stimulated by exo lectrical field stimulation, and th -phenylpiperazinium iodide (D ate preganglionic fibers in the va f ganglia by DMPP or by vagus r old lower concentrations than th r the neuromuscular junction. Th n parasympathetic ganglia is th epression by barbiturates.

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Graf, P. D., Fabbri, L.

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DOSE-DEPENDENT INHIBITION OF COLD AIR-INDUCED BRONCHOCONSTRICTION BY ATROPINE

Inhalation of cold air is a well-known cause of bronchoconstriction in people with asthma. Observations like this have recently triggered a number of investigations dealing with bronchoconstriction induced by cold air or by exercise and inhibited by treatment with atropine or antimuscarinic agents. The study presented here was undertaken to determine whether inhalation of atropine could inhibit cold air-induced bronchoconstriction in a dose-dependent fashion. In seven subjects with asthma the authors assessed the effects of placebo and of various doses of inhaled atropine (0.13-2.08 mg) on base-line specific airway resistance (sRaw) and on the increase in sRaw produced by 5 min of voluntary eucapnic hyperventilation with subfreezing air at −17° C. They also assessed the effect of the lowest dose of atropine on the increase in sRaw produced by five breaths of 1.0% methacholine. Atropine in doses of 0.13 or 0.26 mg caused a maximal reduction in base-line sRaw and completely inhibited the effect of 1.0% methacholine on sRaw, but it did not inhibit the bronchomotor response to cold air. Higher doses of atropine did inhibit the effect of cold air on sRaw in a dose-dependent fashion. The dose of atropine required to inhibit this effect of cold air varied with the increase in sRaw produced by cold air after placebo. These results suggest that cold air causes bronchoconstriction through vagal pathways and that higher doses of antimuscarinic agents may be required to inhibit vagally mediated bronchoconstriction than those required to reduce base-line airway tone or to inhibit the effects of a large dose of an inhaled muscarinic agonist.

Sheppard, D., Epstein, J., Holtzman, M. J., *Nadel, J. A.*, and Boushey, H. A.

Journal of Applied Physiol: Respirat. Environ. Exercise Physiol. 53(1):169-174, 1982.

Other support: National Heart, Lung, and Blood Institute and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco; and Medical Service, San Francisco General Hospital, San Francisco.

BARBITURATES DEPRESS VAGAL MOTOR PATHWAY TO FERRET TRACHEA AT GANGLIA

Barbiturates have been shown before to depress the bronchomotor response to stimulation of the vagus nerve. In the present attempt to determine which site in the vagal motor pathway to airway smooth muscle is most sensitive to depression by barbiturates, isometric muscle tension *in vitro* was recorded and the vagal motor pathway at four different sites before and after exposure to barbiturates was stimulated. In isolated tracheal rings from ferrets, muscarinic receptors in the neuromuscular junction were stimulated by exogenous acetylcholine, postganglionic nerve fibers by electrical field stimulation, and the postsynaptic membrane in ganglia by 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). A tracheal nerve-muscle preparation to stimulate preganglionic fibers in the vagus nerve electrically was also developed. Activation of ganglia by DMPP or by vagus nerve stimulation was depressed by barbiturates at 10-fold lower concentrations than those depressing the activation of postganglionic nerves or the neuromuscular junction. These findings suggest that the postsynaptic membrane in parasympathetic ganglia is the site in the vagal motor pathway most sensitive to depression by barbiturates.

Skoogh, B.-E., Holtzman, M. J., Sheller, J. R., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(1):253-257, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

INTRAVENOUS VERSUS INHALED ATROPINE FOR INHIBITING BRONCHOCONSTRICTOR RESPONSES IN DOGS

In the comparative study presented here, the effect of the muscarinic antagonist atropine sulfate given by two routes, intravenous and inhaled, was assessed. Bronchoconstrictor responses were monitored in anesthetized dogs by determining the increase in total pulmonary resistance before and after increasing doses of atropine and then constructing inhibition dose-response curves. Results showed that there can be a marked difference between the inhibitory effects of atropine on bronchoconstrictor responses to inhaled acetylcholine and to acetylcholine released by vagal stimulation and that the difference depends only on the route of atropine administration. When atropine was administered as an aerosol for inhalation, there was more potent inhibition of the bronchoconstrictor responses to inhaled acetylcholine than of the bronchoconstrictor responses to vagal stimulation. By contrast, when atropine was administered intravenously, there was equivalent inhibition of both stimuli. Therefore, in studies designed to inhibit bronchoconstriction due to an inhaled muscarinic agonist to the same degree as bronchoconstriction due to vagal reflex, atropine might better be given intravenously than by inhalation.

Holtzman, M. J., McNamara, M. P., Sheppard, D., Fabbri, L. M., Hahn, H. L., Graf, P. D., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(1):134-139, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

REGULATORY PEPTIDES IN THE MAMMALIAN RESPIRATORY TRACT

The full respiratory tracts of 11 adult cats, eight Wistar rats, and six Duncan Hartley guinea pigs were isolated and subjected to radioimmunoassay (RIA) and immunocytochemistry in an attempt to map and quantify the presence of five peptides, bombesin, cholecystokinin (CCK), SRIF, substance P, and vasoactive intestinal polypeptide (VIP), in various areas of the respiratory tracts. VIP and substance P were found in higher concentrations than CCK, SRIF and bombesin. In general, higher concentrations were found in the main airways than in lung tissue. Immunocytochemistry localized VIP and substance P to autonomic nerves, whereas bombesin was found in very scattered mucosal endocrine cells of the cat bronchial epithelium. CCK

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Ghatei, M. A., Sheppard, M.
G. P., *Polak, J. M.*, and Bloc

Endocrinology 111(4):1248-125

Other support: Medical Resea

From the Departments of Medi
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OXYGEN-RADICAL-MEDIA VASOCONSTRICTION IN IS

The investigation presente
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Tate, R. M., Vanbenthuysen, K
E.

American Review of Respirator

Other support: Kroc, Hill, Sw
Health, American Heart Associ

From the Webb-Waring Lung In
ter, Denver.

and SRIF could not be convincingly localized by immunocytochemistry because of their low tissue concentrations. In addition, species differences were noted, with high VIP but low substance P concentrations in the main airways of the cat; this relationship was reversed in the rat and guinea pig. Gel permeation chromatography of extracts revealed one peak, coeluting with the pure standard peptide, for CCK-8, SRIF, substance P, and VIP. Bombesin immunoreactivity was separable into two peaks, the major of which corresponded in position to that of gastrin-releasing peptide and a later minor peak to the position of the bombesin standard. Thus, the finding of five potently active peptides in the respiratory tract of these three species strongly suggests that they have a regulatory role in respiratory function. In fact, many recent studies indicate that the respiratory tract has in it the elements of an elaborate peptidergic control system that may conceivably have a significant role in the control of respiratory function in both health and disease.

Ghatei, M. A., Sheppard, M. N., O'Shaughnessy, D. J., Adrian, T. E., McGregor, G. P., *Polak, J. M.*, and Bloom, S. R.

Endocrinology 111(4):1248-1254, 1982.

Other support: Medical Research Council, UK, and the Wellcome Foundation.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London.

OXYGEN-RADICAL-MEDIATED PERMEABILITY EDEMA AND VASOCONSTRICTION IN ISOLATED PERFUSED RABBIT LUNGS

The investigation presented here demonstrates that chemically-generated O_2 intermediates can cause increased alveolar-capillary membrane (ACM) permeability and vasoconstriction in isolated lungs. In order to identify this specific interaction of O_2 radicals and lung tissue, it was necessary to test the effects of O_2 radicals in the absence of other toxic substances such as neutrophil or platelet products. The O_2 radicals generated by xanthine oxidase caused protein-rich edema and increases in lung perfusion pressures that were inhibitable by catalase (hydrogen peroxide scavenger) or dimethylthiourea (hydroxyl radical scavenger) but not by superoxide dismutase. To determine the effect of O_2 radicals on ACM permeability without interference from increased perfusion pressures, papaverine was used to maintain baseline perfusion pressures during O_2 radical exposure and then ACM integrity was assessed by evaluating the response of isolated lungs to elevated outflow pressures (10mm Hg for 10 min). Under these conditions, increased ACM permeability manifested by weight gains and lavage albumin accumulations occurred in lungs treated with xanthine oxidase but not in control lungs. It was concluded, therefore, that O_2 radicals can cause increased ACM permeability and vasoconstriction in isolated lungs.

Tate, R. M., Vanbenthuysen, K. M., Shasby, D. M., McMurtry, I. F., and *Repine, J. E.*

American Review of Respiratory Disease 126:802-806, 1982.

Other support: Kroc, Hill, Swan and Kleberg Foundations, National Institutes of Health, American Heart Association and American Lung Association of Colorado.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

ACETYL GLYCERYL ETHER PHOSPHORYLCHOLINE-STIMULATED HUMAN PLATELETS CAUSE PULMONARY HYPERTENSION AND EDEMA IN ISOLATED RABBIT LUNGS

The occurrence of macrophages, neutrophages, pulmonary platelet microthrombi, and intrapulmonary platelet sequestration in the adult respiratory distress syndrome (ARDS) suggests that platelets may contribute to this form of acute edematous lung injury. Because stimulated human macrophages and neutrophils can release acetyl glyceryl ether phosphorylcholine (AGEPC), a potent platelet activator, it was hypothesized that in ARDS, leukocyte release of AGEPC might stimulate platelets to release thromboxane A_2 (TXA_2), which then produces pulmonary hypertension and lung edema. In support of this premise, the authors found that pulmonary hypertension and edema occurred in isolated rabbit lungs perfused with human platelets and AGEPC, but not with platelets or AGEPC alone. Infusion of a vasodilator (nitroglycerin) to maintain base-line pulmonary artery pressures in lungs perfused with platelets and AGEPC prevented the development of lung edema, suggesting that platelet- and AGEPC-induced edema was hydrostatic in nature. Additional experiments suggested that the increased pressure was a result of TXA_2 release from platelets stimulated by AGEPC. Specifically, preincubation of platelets with imidazole, a thromboxane synthetase blocker, prior to infusion with AGEPC significantly diminished pulmonary hypertension and prevented lung edema. The role of TXA_2 was further suggested when perfusates from lungs infused with platelets and AGEPC developed high levels of TXA_2, whereas perfusates from controls did not. These results suggest that platelet aggregation induced by AGEPC may contribute to ARDS by releasing TXA_2, which raises microvascular pressure and increases edema formation, especially when an underlying permeability defect is present.

Heffner, J. E., Shoemaker, S. A., Canham, E. M., Patel, M., McMurtry, I. E., Morris, H. G., and *Repine, J. E.*

Journal of Clinical Investigation 71:351-357, 1983.

Other support: American Heart Association of Colorado, National Institutes of Health, American Lung Association of Colorado, and Kroc, Hill, Swan, and Kleberg Foundations.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

KINETIC ANALYSIS OF RESPIRATORY TRACT PROTEINS RECOVERED DURING A SEQUENTIAL LAVAGE PROTOCOL

This paper presents an attempt to analyze the dynamic recovery of specific protein substances from the bronchoalveolar space during a sequential lavage protocol. Although bronchoalveolar lavage (BAL) has been used as a research tool for over a decade, the technique of lavage has varied markedly between laboratories. For example, lavage instillate volumes from 50 to 300 ml have been used, and yet the influence of the variable of total lavage volume on subsequent protein recovery is uncertain. In this study, a sequential lavage protocol was performed on 14 normal individuals and the recovered aliquots were analyzed individually for the absolute and relative concentrations of several protein substances. These proteins included free secretory component and secretory IgA, which emanate from airway secretions, and IgG, which is thought

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Merrill, W., O'Hearn, E., F

American Review of Respir

Other support: Veterans Ac

From the West Haven Veter
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Ryan, U. S. and Ryan, J. W.

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Other support: U. S. Public H

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to transude from more distal alveolar sites. Analysis of these data showed a marked decrease in the absolute concentration of all proteins measured in serial aliquots. Analysis of protein ratios in sequential aliquots, however, revealed no significant change from the first to the fifth recovered aliquot. Finally, the influence of the size of the first recovered aliquot on absolute and relative concentrations of proteins was measured. Here there seemed to be a trend indicating preferential recovery of airway proteins in smaller aliquots. This was significant for the ratio of free secretory component to albumin. It was concluded that lung proteins are efficiently and homogenously sampled with 100 ml of lavage instillate. Larger volumes will add more protein but not alter protein ratios. Lavage with smaller volumes may preferentially sample airway proteins.

Merrill, W., O'Hearn, E., Rankin, J., Naegel, G., Matthay, R., and *Reynolds, H. Y.*

American Review of Respiratory Disease 126:617-620, 1982.

Other support: Veterans Administration.

From the West Haven Veterans Administration Medical Center, West Haven, CT, and the Department of Medicine, Yale University, New Haven, CT.

ANGIOTENSIN-CONVERTING ENZYME: II. PULMONARY ENDOTHELIAL CELLS IN CULTURE

One of the technical limitations to progress in understanding of specific functions of a particular cell type is the availability of that cell type in pure culture. Although over the past 12 years a number of different specific metabolic activities have been attributed to pulmonary endothelial cells, by and large these activities were based on conclusions drawn from indirect evidence. In this reported attempt to be able to examine more directly for specific metabolic activities, a program was started to obtain pulmonary endothelial cells in culture. Two methods were developed: (1) cells can be obtained from pulmonary artery and vein of large animals (cow, pig), and (2) cells can be obtained from the microvasculature of small animals (rat, guinea pig, and rabbit). The latter technique can also be used to obtain cells from a lobe of lung from large animals and may be adaptable for use with human tissue. In the first technique, pulmonary arteries, free of blood, are filled with collagenase in Puck's saline for 25 min. The collagenase mixture containing cells is removed and centrifuged; the pellet is then resuspended and seeded into culture flasks. In the second method, lungs are perfused (artery to vein) with Krebs-Henseleit solution until the effluent is blood-free. Collagenase is introduced, and the lungs are then perfused in the opposite direction (vein to artery) until the flow stops spontaneously (ca. 15 min). The detached cells are collected and seeded as before. The cells possess converting enzyme activity and are reactive with antibodies to converting enzyme, Factor VIII and α_2-macroglobulin. The cells synthesize prostaglandins and related substances. In addition, they possess ADPase and synthesize angiotensin-converting enzyme.

Ryan, U. S. and Ryan, J. W.

Environmental Health Perspectives 35:171-180, 1980.

Other support: U. S. Public Health Service and the John A. Hartford Foundation, Inc.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

USE OF MICROCARRIERS TO ISOLATE AND CULTURE PULMONARY MICROVASCULAR ENDOTHELIUM

Among the challenges for endothelial cell culture are (1) development of means of harvesting cells from different tissues and from different sized vessels of a single tissue, and (2) development of means of cell harvest and passage that preserve and do not damage irremediably cell surface structures. The latter has been accomplished for endothelium of large vessels but not for small vessels until now. In the present study, however, means have been devised for collecting and culturing endothelial cells of pulmonary arterioles of approximately 40-60 μm in diameter. The procedure is illustrated by collection of endothelial cells from rabbit pulmonary pre-capillary vessels. The lungs are perfused free of blood with physiological saline and then cold (4°C) saline (containing EDTA and microcarriers 600/ml; 40-60 μm diameter) is perfused via the pulmonary artery. The direction of flow is reversed periodically to collect the bead-cell harvest from the arterial side. Cold shock and EDTA cause the endothelial cells to detach from the vessels under conditions such that the cells remain attached to the microcarriers. The cells are collected on the microcarriers still in the presence of a chelating agent but in the absence of proteolytic enzymes. It is believed that this approach is sufficiently general to allow the collection of endothelium from arterioles and venules of similar size in any tissue. Further, by using microcarriers of different sizes, it should be possible to extend the approach so that one can obtain cultures of endothelia representative of vessels of all sizes.

Ryan, U. S. et al.

Tissue & Cell 14(3):597-606, 1982.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

PROLIFERATION OF PULMONARY ENDOTHELIAL CELLS: TIME-LAPSE CINEMATOGRAPHY OF GROWTH TO CONFLUENCE AND RESTITUTION OF MONOLAYER AFTER WOUNDING

In a regular culture, seeded at low density, all of the cells divide and continue to do so until confluence is reached. Artificial "wounding" (denudation) of a confluent monolayer of pulmonary artery endothelial cells elicits a regenerative response, resulting in the reformation of a continuous single layer of endothelium. Maintenance of this monolayer is important both for the exchange of nutrients and for interactions between blood solutes and endothelial enzymes and transport systems. In the study presented here, time-lapse cinematography was used to compare proliferative behavior of bovine pulmonary endothelial cells in (1) establishment of a monolayer from a low-density seed and (2) restitution of a confluent monolayer following a mechanical wound (removal of cells from an area 5 × 15 mm by scraping). Culture 2 was not refed after wounding. In culture 2, approx. 30% of the cells accounted for repopulation (confluence in 40 hr). In culture 1, all cells entered into division. Participating cells of culture 2 began division immediately (69 divisions/filmed area in 10 hr. *vs.* four divisions in culture 1). Interdivision times (IDT) were longer and relatively constant in culture 1 until near confluence; none were < 10 h, whereas in 2, 24% of the IDT's were ≤ 10 hr. Remarkably, IDTs of culture 2 decreased steadily until confluence was re-

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Ryan, U. S. et al.

Tissue & Cell 14(4):637-

Other support: National

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Ryan, U. S., Whitney, P.

Journal of Appl. Physiol.

Other support: National

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EFFECTS OF BRADYK
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established. Cell migration in culture 1 was multidirectional while direction of migration in culture 2 was always into the wound area. Mean migration rate (MIG) in culture 2 was related to the site of origin of the cells. Neither culture formed more than a single layer of cells. Although the cell kinetics of cultures 1 and 2 differed, the same goal, confluence, was achieved in either case.

Ryan, U. S. et al.

Tissue & Cell 14(4):637-649, 1982.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

LOCALIZATION OF CARBONIC ANHYDRASE ON PULMONARY ARTERY ENDOTHELIAL CELLS IN CULTURE

On the basis of past histological studies, it appears that pulmonary endothelial cells, but not epithelial cells, possess carbonic anhydrase having access to plasma solutes. This work shows specifically that bovine pulmonary artery endothelial cells in culture possess carbonic anhydrase activity and immunoreactivity. Specific immunofluorescence is obtained here when the cells are incubated with rabbit antibovine erythrocyte carbonic anhydrase B and then with goat antirabbit immunoglobulin G coupled to fluorescein. At the level of electron microscopy, antibodies to carbonic anhydrase B are reactive with sites along the plasma membrane and associated caveolae. Multivesicular bodies are the only intracellular sites labeled and appear to correspond to the globular sites of intracellular immunofluorescence. Cells maintained and propagated in culture in the absence of an exogenous source of carbonic anhydrase nonetheless possess carbonic anhydrase activity, suggesting that the cells are capable of synthesizing the enzyme. Taken together, these results indicate that pulmonary artery endothelial cells possess carbonic anhydrase situated so that the enzyme could readily catalyze the dehydration of plasma HCO_3^- to facilitate CO_2 excretion and participate in the regulation of blood pH as central venous blood is converted into systemic arterial blood.

Ryan, U. S., Whitney, P. L., and Ryan, J. W.

Journal of Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53(4):914-919, 1982.

Other support: National Heart, Lung and Blood Institute.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

EFFECTS OF BRADYKININ ON PULMONARY ENDOTHELIAL CELLS IN CULTURE

As a prelude to studies to examine for specific receptors for bradykinin (BK) on pulmonary endothelial cells, these investigators began to examine for the effects of BK on the cyclic nucleotide contents of pulmonary endothelial cells pretreated with a

phosphodiesterase inhibitor. To do this, endothelial cells were harvested from bovine mainstem pulmonary artery and were maintained and passaged in culture. Cells were then prepared for assays, which were performed using kits obtained from New England Nuclear Corp. Angiotensin converting enzyme (ACE) was assayed using [^{3}H]benzoyl-Phe-Ala-Pro as substrate. Results showed an apparent sensitivity of bovine pulmonary artery endothelial cells to BK in concentrations at or near those believed to occur physiologically. Equally impressive was the corresponding finding that BK, but none of its lower homologs, exerted effects on the release of arachidonate or its metabolites. Des-Arg^1-BK, at 10^{-7}M, exerted weak effects. Des-Arg^9-BK was not effective even at 10^{-6}M. Similarly, the products formed by ACE, namely the 1-7 and 1-5 homologs of BK, were completely inactive. Hence, there was remarkable specificity in terms of BK and its metabolites. These and other results make it clear that pulmonary endothelial cells are responsive to BK in reasonable concentration. Thus, it is likely that efforts to examine these cells in culture for high affinity binding sites, characteristic of receptors as opposed to metabolic enzymes, will yield affirmative results.

Ryan, U. S., Lehotay, D. C. and Ryan, J. W.

Advances in Experimental Medicine and Biology 156B:767-774, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Department of Medicine, University of Pittsburgh, Pittsburgh.

A RADIOASSAY FOR CARBOXYPEPTIDASE N

A simple radioassay is described for carboxypeptidase N (CPN), an enzyme that occurs in plasma and on vascular endothelial cells and epithelial brush border. Since CPN has been reported to be a kininase enzyme that participates in the metabolism of one or more of the anaphylatoxins, this report not only describes the assay but looks at some of the ways that the assay can be used further to explore whether CPN is a physiologically important kininase enzyme or merely, in this context, an enzyme capable of degrading bradykinin *in vitro* but not *in vivo*. In one part of this study, CPN was tested for its reactivity with [^{3}H]benzoyl-Phe-Arg. In another part, a series of experiments were performed in which 2-mercaptomethyl-3-guanidinoethylthiopropionic acid (MGP) was added to the reaction mixture as an inhibitor of CPN. Results showed that [^{3}H]benzoyl-Phe-Arg might be a useful substrate for carboxypeptidase N (and presumably carboxypeptidase B). The substrate can be used conveniently in either *in vitro* or *in vivo* assays. When used as described here for *in vivo* assays, hydrolysis is owing primarily to the tissue-bound enzyme (*e.g.*, that on endothelial cells) and not to the soluble enzyme of plasma.

Ryan, J. W., Chung, A. and *Ryan, U. S.*

Advances in Experimental Medicine and Biology 156B:867-874, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

STIMULATED HUMAN I
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From the Hematology-Onco
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STIMULATED HUMAN PHAGOCYTES PRODUCE CYTOGENETIC CHANGES IN CULTURED MAMMALIAN CELLS

It has been known that the occurrence of sister-chromatid exchanges in response to diverse physical and chemical agents correlates strongly with the induction of mammalian mutagenicity or carcinogenicity or both. In the study reported here, it is shown that stimulated phagocytes can cause cytogenetic changes in mammalian cells by means of an oxygen-mediated process. Using the sister-chromatid-exchange assay, it was seen that there was a concentration-dependent increase in sister-chromatid exchanges in cultured Chinese hamster ovary cells incubated with leukocytes activated by phorbol myristate acetate. A particulate activator of oxygen metabolism—opsonized zymosan—was also effective, although not to the same extent. The difference is ascribable to the fact that at the concentrations used in this study, opsonized zymosan is a less potent stimulating agent for leukocytic oxidative metabolism than phorbol myristate acetate. These results prove that leukocyte-generated oxygen metabolites or their reaction products with target-cell components can reach the nucleus of the cultured mammalian cell and cause cytogenetic damage—an observation that may help explain the relation between chronic inflammatory states and cancer *in vivo*.

Weitberg, A. B., Weitzman, S. A., Destrempes, M., Latt, S. A., and *Stossel, T. P.*

The New England Journal of Medicine 308(1):26-30, 1983.

Other support: Elsa U. Pardee Foundation, the Ladies' Auxilary to the Veterans of Foreign Wars, Edwin W. Hiam, the American Cancer Society, and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Clinical Genetics Division, Children's Hospital Medical Center, Harvard Medical School, Boston.

CA^{2+} CONTROL OF ACTIN FILAMENT LENGTH: EFFECTS OF MACROPHAGE GELSOLIN ON ACTIN POLYMERIZATION

Gelsolin, a calcium-binding protein of rabbit lung macrophages, confers calcium sensitivity to the regulation of the network structure of actin filaments. In the presence of micromolar calcium concentrations, gelsolin shortens actin filaments (F-actin) without decreasing appreciably the total mass of filaments, leading to a decrease in the gel structure of actin filaments. Since gelsolin is bound to the shortened filaments, it is likely that gelsolin resides at one or both ends of the filaments to prevent them from annealing. The paper presented here identifies by electron microscopy the end of the actin filament to which gelsolin binds, and shows that gelsolin promotes the nucleation of actin to produce a population of short filaments similar to that obtained when gelsolin is added directly to F-actin. In these respects, gelsolin closely resembles a number of other agents such as the cytochalasins, β-actinin, villin, fragmin, and the *Acanthomoeba castellani* capping protein that have been shown to enhance the nucleation of actin and in some cases to shorten actin filaments, to bind one end of the filaments and to reduce the actin network structure. The identification of such a class of functionally analagous actin-modifying agents suggests that the control of actin filament length is of considerable importance for the regulation of cell structure and movement. As is pointed out here, the calcium-dependent shortening of actin filaments is the primary mechanism for the dissolution of an actin gel by gelsolin. Therefore, the ability of gelsolin to produce short filaments irrespective of the initial state of assembly

of the actin offers flexibility for controlling the network structure of the cytoplasm in which either the monomeric or polymeric form of actin molecules might predominate at different times.

Yin, H. L., Hartwig, J. H., Maruyama, K., and *Stossel, T. P.*

The Journal of Biological Chemistry 256(18):9693-9697, 1981.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

SOME PERSPECTIVES ON THE VISCOSITY OF ACTIN FILAMENTS

The existence of actin filaments at the periphery of many cells has encouraged extensive study of the solution properties of F-actin. In the paper presented here, measurements of the dynamic viscosity of various actin filament preparations under conditions of low and controlled shear: (a) confirm the shear rate dependence of F-actin viscosities and show that this dependence obeys the power law relationship observed for entangled synthetic polymers; (b) permit estimation of the extent to which shear artifact amplifies changes in the apparent viscosity of F-actin measured in a falling ball viscometer; (c) show that gel-filtration chromatography of actin and the addition of cytochalasin B to F-actin bring about small (20-40%) changes in the viscosity of the F-actin solutions. These variations are consistent with alterations in the actin-binding protein concentrations required for incipient gelation, a parameter inversely related to average filament length. Therefore: (a) changes in the viscosity of F-actin can be magnified by use of the falling ball viscometer, and may exaggerate their biological importance; (b) chromatography of actin may not be required to obtain meaningful information about the rheology of actin filaments; (c) changes in actin filament length can satisfactorily explain alterations in F-actin viscosity exerted by cytochalasin B and by chromatography, obviating the need to postulate specific interfilament interactions.

Zaner, K. S. and *Stossel, T. P.*

The Journal of Cell Biology 93:987-991, 1982.

Other support: U. S. Public Health Service and the Whitaker Health Science Fund.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

ACTIN GELATIN AND THE STRUCTURE OF CORTICAL CYTOPLASM

This paper summarizes the evidence, based to a large extent on these investigators' studies of proteins isolated from mammalian phagocytic leukocytes, that the nature of molecules that cross-link actin and the control of the length distribution of actin filaments are profoundly important for the regulation of the consistency of actin filaments *in vitro* and probably *in vivo*. Also summarized here is evidence that the gel structure proposed to exist in the cortex of many cells is pertinent to several physiologic phenomena; one specificially emphasized is cytoplasmic movement. In a section entitled, CONSISTENCY OF ACTIN IN CYTOPLASM, the following parameters are discussed: Rheology of Actin Filaments, Gelation of Actin Filaments, Regulation of the Actin Sol-Gel Transformation by the Control of Filament Length, and Does Capping

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Stossel, T. P. et al.

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Stossel, T. P. et al.

Cold Spring Harbor Symposia on Quantitative Biology 46:569-578, 1982.

Other support: U. S. Public Health Service, the Edwin S. Webster Foundation and Edwin W. Hiam.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

CALCIUM CONTROL OF ACTIN NETWORK STRUCTURE BY GELSOLIN

This book chapter reviews a system which depends on calcium for the control of actin structure. The general principle behind this system is that calcium regulates the *average length* of actin filaments and can thereby control their viscosity and rigidity. The components of this system were first identified in rabbit lung macrophages which can be considered a prototype of highly motile cells. Therefore, this principle of the regulation of cell motility is likely to be applicable in general to other motile eukaryotic cells. In considering the structure of the cortical cytoplasm, it is noted here that the motor of the macrophage appears to reside in the peripheral cytoplasm beneath the plasma membrane. Under light microscopy, the cortical region excludes organelles contributing to the gel-like appearance of the cytoplasm. The thickness of this cortical region changes during various cell activities. Calcium regulation of actin filament length and consequently cytoplasmic rigidity is discussed in a section on regulation of actin gel-sol transformation. Another section is devoted to calcium regulation of actin filament by gelsolin, a 91,000-dalton, heat-labile, globular protein, which shortens actin filaments when activated by calcium in the micromolar concentration range contributing to the collapse of their three-dimensional lattice. When the ambient calcium concentration is decreased, the regulator is inactivated and the actin gel reforms. Overall, information currently available permits a biochemical explanation of calcium-regulated directional movements of the cytoplasm. Calcium activates a regulatory protein, gelsolin, shortening actin filaments. This causes a localized breakdown in actin structure, creating a gradient of cytoplasmic rigidity, and directional movement follows from the resultant imbalance in tension generated by myosin.

Yin, H. L. and *Stossel, T. P.*

In: Cheung, Y. W. (ed.): *Calcium and Cell Function*, New York: Academic Press, 1982, Vol. 2, pp. 325-337.

Other support: U. S. Public Health Service, Edwin S. Webster Foundation, Edwin W. Hiam, and Charles L. and Jane D. Kaufman.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

FLOW-VOLUME CURVES IN INFANTS WITH LUNG DISEASE

This study was undertaken to investigate the feasibility of using a new partial expiratory flow-volume (PEFV) technique in infants with a variety of lung diseases and to determine whether such measurements provide additional information on the severity of lung disease, site of obstruction and response to treatment. As reported here, PEFV maneuvers have been performed on nine occasions on six infants with a variety of pulmonary problems using the new technique for thoracic compression. The infants were placed in an inflatable bag that was itself within a canvas bag. By sudden controlled inflation of the inner bag at end inspiration, PEFV curves were generated and recorded by means of a face mask and pneumotachograph. By comparing these flow results with airway resistance and lung volume measurements obtained from the infants in whole body plethysmography and by noting the effect of inhaling a helium/oxygen gas mixture, it was possible to partition the airway obstruction between large and small airways. The presence of small airway obstruction was noted in the absence of changes in airway resistance or lung volume in several instances. A complete evaluation of airway function should include this test of forced expiration for greater understanding and treatment of lung disease in infancy.

Godfrey, S., Bar-Yishay, E., Arad, I., Landau, L. I., and *Taussig, L. M.*

Pediatrics 72(4):517-522, 1983.

Other support: Fogarty International Scholarship and Glaxo Group Research.

From the Department of Pediatrics, Hadassah University Hospital, Jerusalem.

SYNTHETIC ELASTASE INHIBITORS: PROSPECTS FOR USE IN THE TREATMENT OF EMPHYSEMA

Human leukocyte (HL) elastase is considered to be the enzyme primarily responsible for the destruction of lung tissue observed in pulmonary emphysema. In the review paper presented here, several approaches to treatment of emphysema are discussed. These include (1) use of α_1-Protease Inhibitor (α_1-PI), (2) use of other natural high molecular weight protease inhibitors from either plant or animal sources, (3) use of antioxidants to protect the α_1-PI, and (4) use of low molecular weight elastase inhibitors. Recent research has shown that synthetic elastase inhibitors can be designed to bind either reversibly or irreversibly to elastase. Indeed, a number of potent reversible and irreversible inhibitors have already been developed for human leukocyte elastase. Several of these inhibitors have been shown to be effective at preventing emphysema in animal models of the disease. As of now it is clear that a synthetic elastase inhibitor would be a useful therapeutic agent and that there are excellent prospects for its development for use in treating human emphysema.

Powers, J. C. (*Travis, J.*)

American Review of Respiratory Disease 127:S54-S58, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

IMMUNOLOGIC MEASUR
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IMMUNOLOGIC MEASUREMENT OF ELASTIN-DERIVED PEPTIDES IN HUMAN SERUM

Chronic obstructive pulmonary disease (COPD) develops insidiously over many years, and usually significant impairment of lung function has occurred before the disease is diagnosed. It seems possible that a quantitative test that is capable of identifying the presence of lung damage at an early stage, before symptoms develop, could be useful in identifying those people who are at risk and possibly in monitoring the progression of the disease. It is likely that destruction of the elastin fiber is a prerequisite for the development of COPD, and it is possible that immunologic identification in the serum of peptides derived from lung elastin degradation might be an effective approach to the early detection and monitoring of the disease. These investigators prepared antibodies to peptides derived from human lung parenchymal elastin and used these antibodies in an enzyme-linked immunosorbent assay to quantitate elastin-derived peptides in the serum of 39 normal control nonsmokers, 33 smokers with normal lung function and 40 patients with COPD. On average, statistically significant higher levels of elastin-derived peptides were found in the normal smokers and COPD patients compared to the controls. Further work with large numbers of subjects is necessary to determine whether such a test could be effective in identifying those individuals who are at risk of developing COPD.

Kucich, U., Christner, P., Lippmann, M., Fein, A., Goldberg, A., Kimbel, P., *Weinbaum, G.*, and Rosenbloom, J.

American Review of Respiratory Disease 127:S28-S30, 1983.

Other support: National Institutes of Health.

From the Pulmonary Disease Section, Albert Einstein Medical Center, Philadelphia; the Center for Oral Health Research, Department of Anatomy and Histology, School of Dental Medicine, University of Pennsylvania, Philadelphia; and the Department of Medicine, the Graduate Hospital, Philadelphia.

DYNAMICS OF THE NEUROENDOCRINE CELL—REGULATORY PEPTIDE SYSTEM IN THE LUNG: SPECIFIC OVERVIEW AND NEW RESULTS

This overview of the biology of the lung neuroendocrine (NE) cells includes some review material from earlier studies and certain new observations dealing with oxygen-related changes in NE cell numbers. For the study presented here, changes in argyrophil NE cell numbers, intensity of 5-HT fluorescence, and arterial medial thickness in the lungs of neonate rabbits were assessed under various oxygen treatments. Results of this study showed that NE cell numbers and 5-HT fluorescence in normal rabbits increased from 12 hours before to one day after birth, and NE cells declined thereafter to the 10th day. In acute hypoxic 5-day-old rabbits, 5-HT fluorescence was decreased, whereas NE cell numbers and medial thickness were unchanged. Neonates hypoxic from birth had higher NE cell numbers and increased medial thickness at three and five days, whereas 5-HT fluorescence was decreased compared to normoxic controls. These chronically hypoxic neonates showed a dramatic drop in argyrophil NE cell numbers to below normal when they were exposed to normoxia for one hour, but cell numbers and medial thickness returned to normal at 4 and 24 hours, respectively. The effect of acute and chronic hyperoxia was also tested and results showed that 100% 0_2 for 2-2.5 hours caused a significant drop in detectable NE cell numbers, whereas 40% 0_2 in N_2 caused no change. Although at the present time the mechanisms and pathways

that tell us how the biogenic amines and polypeptide hormones function in lung control cannot be defined, the accumulated evidence that these substances are present and active implies that their role is vital.

Keith, I. M. and *Will, J. A.*

Experimental Lung Research 3(3&4):387-402, 1982.

Other support: College of Agricultural and Life Sciences, University of Wisconsin-Madison and the Air Force Office of Scientific Research.

From the Department of Veterinary Science, CALS, and the Department of Anesthesiology, Medical School, University of Wisconsin-Madison.

III. *Heart and Circulation*

DISTRIBUTION OF HIGH DENSITY LIPOPROTEIN PARTICLES WITH DIFFERENT APOPROTEIN COMPOSITION: PARTICLES WITH A-I AND A-II AND PARTICLES WITH A-I BUT NO A-II

High density lipoproteins (HDL) have been shown to contain numerous chemically and immunochemically distinct polypeptides referred to as apoproteins (apo). In the study reported here, HDL were subfractionated by equilibrium CsCl gradient centrifugation of the d 1.063-1.21 g/ml HDL fraction isolated from two men and two women. The various HDL subfractions were analyzed for their apo A-I, A-II, B, D, and E and the major lipid contents. ApoA-I and A-II were found throughout the density gradient. ApoE was found in all HDL fractions with the higher concentration in the lower density fractions. Conversely, the concentration of apoD increased as the density of the HDL fraction increased. Each density subfraction underwent quantitative precipitation with anti-A-I and anti-A-II immunoglobulin. Essentially all A-II in all density subfractions was precipitated with either immunoglobulin. Particles from each density subfraction precipitated with anti-A-II immunoglobulin had an A-I/A-II molar ratio of approximately 2.0 (range 1.9-2.3). However, particles precipitated with anti-A-I immunoglobulin had A-I/A-II molar ratios identical to the A-I/A-II ratio of the subfraction (range 2.1-7.1). The subfractions with A-I/A-II molar ratios of about 2 had the least proportion of A-I in particles containing A-I but not A-II. Conversely, the subfractions with the highest A/I/A-II molar ratio had the greatest proportion of apoA-I in particles containing A-I but not A-II. These data indicate that HDL contains at least two types of particles: particles with both A-I and A-II in a 2:1 molar ratio, and particles containing A-I but no A-II. The variation in A-I/A-II ratio observed in different HDL density subfractions was due to the different proportions of these types of particles.

Cheung, M. C. and *Albers, J. J.*

Journal of Lipid Research 23:747-753, 1982.

Other support: National Institutes of Health.

From the Northwest Lipid Research Clinic and Department of Medicine, University of Washington School of Medicine, Seattle.

CULTURED ENDOTHEL
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Medicine, University of

CULTURED ENDOTHELIAL CELLS DERIVED FROM THE HUMAN ILIAC ARTERIES

The purpose of this cell culture study was to establish *in vitro* cell cultures derived from the endothelium of human great arteries from individuals with varying degrees of atherosclerotic disease. To do this, cells derived from the endothelium of human iliac arteries were cultured *in vivo*. The cells were isolated, grown and subcultured in HEPES buffered Medium 199 supplemented with 20% heat inactivated human whole blood serum, human alpha-thrombin, and commercial endothelial cell growth supplement derived from bovine brain. The cells were viable in culture for 8 to 10 passages at a split ratio of 1:3. After the 10th passage, the cells began to enlarge and their growth rate was reduced. No cultures were viable after the 12th passage. The cells were determined to be of endothelial origin by their morphology at confluence, their ultrastructural characteristics, the production and release of factor VIII-related antigen, and by their maintenance of a surface that prevented platelet attachment. The cultured arterial endothelial cells released prostacyclin in response to challenge with thrombin and protamine sulfate, but not in response to bradykinin or the platelet-derived growth factor. Although the cultures described here were derived from patients with varying degrees of atherosclerotic disease, there were no significant differences in morphological or physiological parameters among the cultures or in comparison with commonly studied cells derived from human umbilical veins.

Glassberg, M. K., Bern, M. M., Coughlin, S. R., Haudenschild, C. C., Hoyer, L. W., *Antoniades, H. N.*, and Zetter, B. R.

In Vitro 18(10):859-866, 1982.

Other support: National Institutes of Health.

From the Department of Physiology and Surgery, Children's Hospital Medical Center, Harvard Medical School, Boston; Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston; Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; Mallory Institute of Pathology, Boston University School of Medicine, Boston; Department of Medicine, University of Connecticut Health Center, Farmington; Center for Blood Research and Harvard School of Public Health, Boston.

INCORPORATION OF 7-KETOCHOLESTEROL BY PLASMA MEMBRANES FROM PORCINE AORTIC ENDOTHELIAL CELLS

In this paper evidence is presented that 7-ketocholesterol (7KC) is incorporated into the plasma membranes of endothelial cells in excised porcine aortas in molar amounts comparable to the native cholesterol present in the membrane. These experimental findings indicate that simple adsorption of 7KC does not play a role in the results obtained. Instead 7KC appears to be inserted into the lipid bilayer of the membranes. This is largely independent of membrane cholesterol content and is not due to substitution of the native membrane cholesterol. A significant increase in the total sterol-to-phospholipid ratio is obtained with 7KC-exposure without significant changes in the cholesterol-to-phospholipid ratio. In summary, therefore, these data suggest that the net incorporation of substantial amounts of 7KC in the plasma membranes is very likely due to a process of insertion of the 7KC molecules into the lipid bilayer matrix of the plasma membrane.

Santillan, G. G., Zak, I. and *Bing, R. J.*

Artery 11(2):119-135, 1982.

Other support: The Hoover Foundation.

From the Huntington Memorial Hospital, Huntington Medical Research Institutes, and the California Institute of Technology, Pasadena.

MICROCIRCULATION OF LEFT ATRIAL MUSCLE, CEREBRAL CORTEX AND MESENTERY OF THE CAT: A COMPARATIVE ANALYSIS

Capillary morphometry and topography and O_2 supply to the tissue are intimately related to the capillary functions of maintaining tissue oxygenation and responding rapidly to changing oxygen demands. To investigate these associations, comparative analyses were carried out by means of transillumination on the geometry, topography and morphometry of microcirculation in the cerebral cortex, left atrial muscle and mesentery of the cat using computer analysis. In addition, specific types of capillary distribution (concurrent, countercurrent and asymmetric distribution) in these three organs were ascertained from images visualized on films. These parameters were related to their role in tissue oxygen supply. It was found that mean capillary diameter, mean intercapillary distance, total capillary length, and total capillary surface area differed significantly among the three organs. Differences in mean capillary tortuosity between cerebral cortex and left atrial muscle and between left atrial muscle and mesentery also were significant. Mean capillary tortuosity in mesentery and cerebral cortex was of equal magnitude. In the cerebral cortex a high degree of tortuosity and asymmetric capillary distribution favor tissue oxygenation. A similar situation exists in left atrial muscle. In the mesentery, the combination of high capillary tortuosity and concurrent capillary arrangement is unfavorable for tissue oxygenation.

Chang, B-L., Yamakawa, T., Nuccio, J., Pace, R., and *Bing, R. J.*

Circulation Research 50(2):240-249, 1982.

Other support: The Hoover Foundation.

From the Huntington Memorial Hospital, Huntington Institute of Applied Medical Research, and the California Institute of Technology, Pasadena.

EFFECT OF ALCOHOL ON THE HEART AND CARDIAC METABOLISM

This report was compiled to delineate the disturbances in biochemistry of heart muscle when exposed to ethanol. The disturbances are many. All elements of cellular substructure are effected. In plasma membranes, $(Na^+ + K^+)$-activated ATPase (EC 3.6.1.3) is inhibited. Mitochondrial damage consists of diminished respiratory function and calcium uptake and binding. High-energy phosphates remain intact. Alcohol also effects the malate-aspartate shuttle. Acetaldehyde, a metabolite of ethanol, has a direct effect on myocardial protein synthesis through microsomal inhibition; however, the development of cardiac hypertrophy is not affected. Malfunction of sarcoplasmic reticulum is evidenced by disturbances in calcium binding and uptake. Effects of ethanol on the contractile machinery are deficiencies in the turnover rate of chemical into mechanical energy (diminished V_{max}), and in the number of cross-bridges formed (P_o). It increases stiffness of series elastic elements. There is diminished fatty acid

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Bing, R. J.

Federation Proceedings 41:2

Other support: U. S. Public

From Huntington Memorial
Research, Pasadena, CA.

INHIBITORY EFFECT OF
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Bing, R. J.

Federation Proceedings 41:2443-2446, 1982.

Other support: U. S. Public Health Service and The Hoover Foundation.

From Huntington Memorial Hospital and Huntington Institute of Applied Medical Research, Pasadena, CA.

INHIBITORY EFFECT OF A CALCIUM ANTAGONIST (DILTIAZEM) ON AORTIC AND CORONARY CONTRACTIONS IN RABBITS

The *in vitro* experiments reported here were carried out on 18 coronary arterial and 50 aortic strips from 26 New Zealand white rabbits in an attempt to investigate the effect of a slow calcium channel blocker, diltiazem, on the isometric contractions induced by certain prostaglandins, $PGF_{2\alpha}$ and PGE_2, 5-hydroxytryptamine (5-HT) and nicotine on the isolated strips. Results showed that diltiazem inhibited contraction induced by 5-HT and by the two prostaglandins $PGF_{2\alpha}$ and PGE_2 in coronary arteries only. Specifically these experiments demonstrate that a slow calcium channel blocker, diltiazem, effectively inhibits contractions of coronary artery strips induced by $PGF_{2\alpha}$, PGE_2 and 5-HT and also effectively diminishes the isometric contractions induced by nicotine in aortic strips. The contractions induced by nicotine, which was effective in aortic smooth muscle only, are also markedly inhibited by diltiazem as well as by prozosin an α_1 adrenergic inhibitor. Rauwolscine, an α_2 inhibitor, has little effect.

Sato, M., Ohashi, M., Metz, M. Z., and *Bing, R. J.*

Journal of Molecular and Cellular Cardiology 14(12):741-744, 1982.

Other support: The Hoover Foundation.

From Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

MICROCIRCULATION IN MYOCARDIUM AND CEREBRAL CORTEX

The cerebral microcirculation has been studied by a variety of methods. While most of the early findings were based on histological observations, recently more pertinent information of cerebral microcirculatory flow has been noted by direct observation. However, most of these determinations have been made on the pial vessels by epiillumination. In contrast, few *in vivo* observations of the intracortical cerebral microvasculature have been reported. The report presented here is concerned with findings based on a technique which permitted visualization of the cortical microcirculation in the live anesthetized cat. The following subjects were studied: I. Capillary distribution, II. Comparative analysis of microcirculation in heart and brain, and III. The effect of changes in systemic blood pressure induced by hemorrhage on microcirculatory autoregulation in the cerebral cortex. Many references are considered here and much calculation is presented and carried out. For calculation of capillary

morphometry and geometry, a computer program was designed to run on PDP 11-03 minicomputers. Results of several studies show that there may be a significant interaction between certain types of capillary curving and characteristics of formed blood elements. It is conceivable that more rigid cells may resist any further deformation in narrow capillary curves. The resulting pressure differential affects not only red cell velocity within the same capillary segment, but causes instantaneous redistribution of pressure gradients in adjoining capillaries. An important observation from the comparative analysis of microcirculation in the myocardium, cerebral cortex and mesentery is that capillary morphometry and topography and O_2 supply to the tissue are intimately related.

Bing, R. J. and Chang, B-L.

In: Dintenfass, L., Julian, D. G. and Seaman, G. V. F. (eds.): *Heart Perfusion, Energetics, and Ischemia*, New York: Plenum Publishing Corporation, 1983, pp. 157-178.

Other support: The Hoover Foundation.

From the Department of Experimental Cardiology, Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE ACTION OF DILTIAZEM ON VASCULAR SMOOTH MUSCLE AND ON PROTECTION OF THE ISCHEMIC (DOG) AND ISCHEMIC REPERFUSED (RAT) HEART

This article, which was dedicated to Dr. Albert Fleckenstein on the occasion of his 65th birthday, is devoted primarily to the effects of diltiazem, a calcium antagonist, on vascular smooth muscle of rabbits, and to the drug's effect in protecting the ischemic and ischemic reperfused myocardium. This article is very apropos since Fleckenstein in 1969 was the first to point out that Isoptin® prevents myocardial necrosis induced by isoproterenol. He mentioned for the first time the possible role of Ca^{++} antagonism: "the future will show whether or not there is a rational way to prevent the mechanism of necrosis formation in myocardium by means of calcium antagonists." The results presented here bear out this prediction. In summarizing the material in this paper, the prediction of Fleckenstein in 1968 that the vascular smooth cell is highly susceptible to the action of Ca^{++} antagonists has been proved by the antagonistic effect of Diltiazem on the prostaglandins $PGF_{2\alpha}$ and PGE_2. These findings of the protection of the ischemic and ischemic reperfused myocardium also are in line with the original idea of Fleckenstein, first proposed in 1968: Ca^{++} antagonists prevent myocardial damage by protecting the heart muscle from excessive activation of Ca-dependent intracellular ATP-ases and from Ca^{++} overload. This ingenious view expressed in the sixties, which for the first time stressed the significance of myocardial Ca^{++} overload, serves as a valid explanation of the protection of the ischemic and ischemic reperfused myocardium.

Bing, R. J. and Chang, B-L.

In: *Fleckenstein's 65th Birthday Celebration Volume*, New York: Gustav Fischer Verlag, 1983, pp. 5-13.

Other support: The Hoover Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

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Other support: National

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ALTERATION IN PLA
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BIOLOGICAL SIGNIFICANCE OF HEPARAN SULFATE PROTEOGLYCANS

Heparan sulfate chains have been found to be present as protein-bound carbohydrate chains both at the cell surface and in the supernatant growth medium of rabbit endothelial cultures, thus raising the possibility that these cell products may play a role in two of the most typical functions of the endothelium, the regulation of the exchanges between blood and tissues and the ability to maintain blood fluidity. In earlier experiments, several lines of evidence seem to imply that the heparan sulfate proteoglycans of these cells possess several distinct features that differentiate them from similar molecules synthesized by other cells of the vessel wall. Now, the overall studies considered in this paper suggest that an endothelial cell inhibitor (presumably one of the slower-moving heparan sulfate proteoglycans) interacts with one of the coagulation enzymes of the thromboplastin pathway and by so doing inhibits the conversion of prothrombin to thrombin. Through this mechanism, heparan sulfate proteoglycans may play an essential role in imparting the property of blood compatibility to the endothelial cell surface. The function of the other heparan sulfate species (the electrophoretically faster-moving) is at present unknown. It can be hypothesized that, given the multiplicity of structures that these cell products exhibit, they participate in a wide range of endothelial cell functional activities by either inhibiting or activating enzymes that are an important part of certain blood homeostatic mechanisms or by binding certain proteins of biological importance that influence the functions of the endothelium or its interaction with other blood components. Thus, the generic name of "proteolgycans" comprises a number of species whose structures and functions may be widely different.

Buonassisi, V. and Colburn, P.

Annals of the New York Academy of Sciences 401:76-84, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Department of Biology, University of California at San Diego, La Jolla.

ALTERATION IN PLASMA PROTEINS AND PLATELET FUNCTIONS WITH AGING AND CIGARETTE SMOKING IN HEALTHY MEN

A longitudinal clinical study was started several years ago to investigate the effect of normal aging and chronic smoking on blood coagulation and platelet function. The volunteer subjects used for this study were obtained through the computer research facility of the Normative Aging Study of the Veterans Administration Outpatient Clinic in Boston. As reported here, blood samples were obtained on four different occasions from 18 cigarette smoking and 34 nonsmoking healthy men (age 40-69) and analyzed to assess age-and smoking-associated changes in plasma proteins, blood coagulation and platelet functions. Results showed that collagen-induced platelet aggregation was significantly increased with aging in non-smokers. Significant changes in chronic smokers were increases in platelet count and fibrinogen in plasma; elevation of platelet factor-3 (PF-3) activity in platelet-poor plasma (PPP); increase in serum levels of α_1-antitrypsin, orosomucoid, haptoglobin and properdin factor B; and shortening of the lag period of collagen-induced platelet aggregation. Filtration of PPP through Millipore filters removed PF-3 membranes. The differences in PF-3 activities

in filtered plasma were no longer significant between smokers and nonsmokers. Results suggest that chronic smokers have higher levels of acute phase proteins reflecting underlying inflammatory processes, and higher levels of PF-3 activity in plasma due to liberation of PF-3 membranes from platelets.

Chao, F. C. et al.

Thrombosis and Haemostasis 47(3):259-264, 1982.

Other support: National Institutes of Health and the VA Medical Research Service.

From the Center for Blood Research, Departments of Medicine and Pediatrics, Harvard Medical School, and the Veterans Administration Outpatient Clinic, Boston.

EVIDENCE FOR CALCIUM-SENSITIVE STRUCTURE IN PLATELET THROMBOSPONDIN

The isolation of thrombospondin in the presence of calcium is reported in this paper, where it is also shown that thrombin and trypsin proteolysis, as well as the sedimentation coefficient, intrinsic viscosity, and electron microscopic appearance of thrombospondin, are sensitive to the presence of calcium. As shown here, when thrombospondin, which was purified in the presence of EDTA, was exposed to thrombin, high molecular mass fragments were observed at 175,000, 160,000, 145,000, and 135,000 daltons. In contrast, when the supernatant from thrombin-treated platelets was incubated with additional thrombin, only the 160,000-dalton fragment of thrombospondin was produced. The results of thrombin digestion of supernatant samples under varying ionic conditions suggested that the addition of EDTA during isolation of thrombospondin disrupted native calcium-sensitive structure in thrombospondin. To preserve these structures, thrombospondin was purified by heparin affinity chromatography. The peptide pattern produced by thrombin digestion of purified thrombospondin is different from that observed for thrombospondin in the supernatant from thrombin-treated platelets. Dialysis experiments indicate that the peptide pattern produced by thrombin is dependent upon the presence of calcium. Other results presented here are consistent with low angle rotary shadowing data, which indicate that thrombospondin appeared to be comprised of three to four well defined nodular domains connected by thinner flexible regions.

Lawler, J., *Chao, F. C.* and Cohen, C. M.

The Journal of Biological Chemistry 257(20):12257-12265, 1982.

Other support: National Institutes of Health.

From the Department of Research, St. Elizabeth's Hospital of Boston, Tufts University School of Medicine, Boston, and the Center for Blood Research, Boston.

ASSAY OF PREKALLIKREIN IN HUMAN PLASMA: COMPARISON OF AMIDOLYTIC, ESTEROLYTIC, COAGULATION, AND IMMUNOCHEMICAL ASSAYS

This methodological study compares three functional assays for prekallikrein using coagulation, esterolytic and amidolytic substrates with a radial immunodiffusion

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Fisher, C. A., Schmaier

Blood 59(5):963-970, 19

Other support: National

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FACTOR XI ANTIGEN

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1982.

of Boston, Tufts Univer-
Research, Boston.

COMPARISON OF
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d assays for prekallikrein
n a radial immunodiffusion

reference assay in three subject populations—normals, women receiving oral contraceptives and patients with severe liver disease. The amidolytic assay, which was designed to measure prekallikrein in plasma, uses the substrate H-D-Pro-Phe-Arg-p-nitroaniline-HCl. At a substrate concentration of 1mM, the amidolysis of purified kallikrein at 1 coagulant unit/ml was observed to be 2.47 μmole/min/ml. Conditions for plasma kallikrein activation were optimized to approach complete activation when compared to the amidolytic activity of the purified plasma kallikrein. Activated plasma prekallikrein had 78% of activity of purified kallikrein at plasma concentration. Comparison of this amidolytic assay with immunochemical, esterolytic, and coagulant assays of the three subject populations showed good correlation both in normals and in the patient groups between the amidolytic and esterolytic assays. Each enzymatic assay correlated with the immunochemical assay. However, comparison of each of these assays with the coagulant assay showed no significant correlation due to the large inherent error of the latter assay. This standardized plasma prekallikrein amidolytic assay should facilitate studies of plasma prekallikrein concentration in physiologic and pathologic conditions and help identify activation of the contact phase of coagulation in disease states.

Fisher, C. A., Schmaier, A. H., Addonizio, V. P., and *Colman, R. W.*

Blood 59(5):963-970, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center and the Department of Medicine, Hematology/Oncology Section, Temple University Health Sciences Center, Philadelphia, and the Division of Cardiothoracic Surgery, Department of Surgery, University of Pennsylvania School of Medicine, Philadelphia.

FACTOR XI ANTIGEN AND ACTIVITY IN HUMAN PLATELETS

Previous studies have shown that factor XI activity resides in the plasma membrane fraction, is not inactivated by anti-factor-XI antibody and is present in platelets from a hemostatically normal patient with congenital absence of plasma factor XI. In the present attempt to further explore the nature of the intrinsic factor XI activity of platelets, washed platelets, contaminated with less than 0.20% plasma factor XI, were examined for the presence of factor XI antigen and activity. These platelets contained a factor XI-like coagulant activity that remained constant after successive washes. By means of indirect immunofluorescence, a monospecific antibody to factor XI showed specific staining of both normal platelets and platelets from patients deficient in plasma factor XI. Radiolabeled Triton extracts of washed platelets and labeled purified factor XI solutions were analyzed for factor XI antigen by Staph A immunoprecipitation analysis using antibody to purified plasma factor XI followed by SDS gel electrophoresis. On unreduced gels, the platelet material ran as a single band having an apparent molecular weight of 220,000 daltons, whereas purified plasma factor XI gave a single band at 160,000 daltons. On reduced gels, the platelet material analyzed as a single band at 52,000 daltons, whereas purified factor XI gave a single band of 80,000 daltons. These and other results support the view that platelet factor XI is functionally and antigenically similar to plasma factor XI but different in molecular weight and isoelectric point. In addition, the presence of factor XI activity and antigen in the

platelets of three hemostatically normal individuals with no detectable plasma factor XI activity or antigen supports the conclusion that the platelet material is not of plasma origin and that it may substitute for plasma factor XI in hemostasis.

Tuszynski, G. P., Bevacqua, S. J., Schmaier, A. H., *Colman, R. W.*, and Walsh, P. N.

Blood 59(6):1148-1156, 1982.

Other support: Department of Health, Education and Welfare.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECT OF HEPARIN ON THE INACTIVATION RATE OF HUMAN FACTOR XIa BY ANTITHROMBIN-III

The contribution of heparin toward the inhibition of factor XIa by antithrombin III in purified systems and in plasma was assessed here using amidolytic and coagulant assays. At therapeutic heparin concentrations (1U/ml), no potentiating effect on this reaction was found, although inhibition of the amidolytic activity of thrombin by purified antithrombin-III was enhanced at least 20-fold by the same concentration of heparin. Furthermore, despite the ability of heparin (1U/ml) to increase the inactivation rate of thrombin by plasma, no acceleration of the rate of inhibition of factor XIa by plasma was observed. Similar results were found when the inhibition of factor XIa was monitored with a coagulant assay after first removing heparin. Only at heparin concentrations of 5 and 10 U/ml, was a 2-and 4-fold increase in the inactivation rate of factor XIa by purified antithrombin III observed. Therefore, in both purified systems as well as plasma, heparin, at concentrations observed in clinical practice, does not accelerate the inactivation rate of human factor XIa by antithrombin-III.

Scott, C. F., Schapira, M. and *Colman, R. W.*

Blood 60(4):940-947, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Hematology/Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

PREKALLIKREIN ACTIVATION AND HIGH-MOLECULAR-WEIGHT KININOGEN CONSUMPTION IN HEREDITARY ANGIOEDEMA

Three unrelated patients with hereditary angioedema were studied in this attempt to determine whether activation of the contact phase of coagulation could be detected in peripheral blood during acute attacks of this disease. It has been known before that patients with hereditary angioedema lack $C\bar{1}$ inhibitor, a plasma α_2-glycoprotein that inhibits both the proteolytic action of $C\bar{1}$, the activated first component of the complement system, and the activity of components of the contact phase of coagulation: kallikrein, factor XI_a, and factor XII_a. Such patients have been shown to have low levels of C4 and C2, the natural substrates for $C\bar{1}$, but the levels were not correlated with the presence of symptoms. In the three-patient study presented here, it was found

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Schapira, M., Silver, L. D.,
J. G., and *Colman, R. W.*

The New England Journal o

Other support: Swiss Natior
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From the Thrombosis Rese
University School of Medici
dation, La Jolla, CA.

PURIFIED HUMAN PLAS
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PMN aggregation by plasma

Schapira, M., Despland, E.,

Journal of Clinical Investig

Other support: National Inst
the Riley Memorial Associa

From the Division de Rhum
zerland; Temple University
of Pediatric Hematology-
Indianapolis.

that during a symptomatic period the patients had decreased levels of prekallikrein, a substrate for the activated forms of factor XII, and reductions in high-molecular-weight kininogen, a substrate for plasma kallikrein. These observations suggest that zymogens of the contact system are activated during attacks of hereditary angioedema and that some of the clinical manifestations may be mediated through products of this pathway, such as kinins.

Schapira, M., Silver, L. D., Scott, C. F., Schmaier, E. H., Prograis, L. J., Jr., Curd, J. G., and *Colman, R. W.*

The New England Journal of Medicine 308(18):1050-1054, 1983.

Other support: Swiss National Science Foundation, American Heart Association and the National Institutes of Health.

From the Thrombosis Research Center and the Department of Medicine, Temple University School of Medicine, Philadelphia, and Scripps Clinic and Research Foundation, La Jolla, CA.

PURIFIED HUMAN PLASMA KALLIKREIN AGGREGATES HUMAN BLOOD NEUTROPHILS

The effect of highly purified human plasma kallikrein on polymorphonuclear leukocytes (PMN) is described in this paper, using cell aggregation and oxygen consumption as criteria for neutrophil activation. Results of these studies show that exposure of human blood PMN to purified active plasma kallikrein resulted in PMN aggregation when kallikrein was present at concentrations ranging from 0.4 to 0.6 U/ml (0.18-0.27 μM). Kallikrein-induced PMN aggregation was not mediated through C5-derived peptides, because identical responses were observed whether or not kallikrein had been preincubated with an antibody to C5. Moreover, kallikrein was specific for aggregating PMN, because no aggregation was observed with Factor XII active fragments, Factor XIa, thrombin, plasmin, porcine pancreatic elastase, bovine pancreatic chymotrypsin, or bradykinin. Bovine pancreatic trypsin (2 μM) aggregated PMN, but to a lesser extent than kallikrein (0.18 μM). Kallikrein was a potent aggregant agent for PMN because similar responses were observed with kallikrein (0.5 U/ml or 0.23 μM) and an optimal dose (0.2 μM) of N-formyl-methionyl-leucyl-phenylalanine. In addition, PMN incubation with kallikrein resulted in stimulation of their oxidative metabolism as assessed by an increased oxygen uptake. Neutropenia and leukostasis observed in diseases associated with activation of the contact phase system may be the result of PMN aggregation by plasma kallikrein.

Schapira, M., Despland, E., Scott, C. F., Boxer, L. A., and *Colman, R. W.*

Journal of Clinical Investigation 69:1199-1202, 1982.

Other support: National Institutes of Health, Swiss National Science Foundation and the Riley Memorial Association.

From the Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Temple University School of Medicine, Philadelphia, and the Division of Pediatric Hematology-Oncology, Indiana University School of Medicine, Indianapolis.

HIGH-MOLECULAR WEIGHT KININOGEN: A SECRETED PLATELET PROTEIN

This study presents immunochemical evidence for the presence of high-molecular weight kininogen (HMWK) in platelets and for HMWK's secretion following platelet activation with a divalent cationophore A23187, collagen, and thrombin. In the first place, human platelets were studied immunochemically to determine if they contain HMWK. On crossed immunoelectrophoresis with total kininogen antisera (antisera that recognizes both high- and low-molecular weight kininogen) extracts of platelets contained total kininogen antigen. Platelet total kininogen antigen showed complete antigenic identity with plasma total kininogen and displayed the same electrophoretic migration as plasma total kininogen. Using antisera monospecific to HMWK, a competitive enzyme-linked immunosorbent assay (CELISA) was developed to directly measure platelet HMWK. By CELISA, 27-101 ng of HMWK antigen per 10^8 platelets were quantitated in detergent-soluble lysates of washed human platelets from nine normal donors with a mean level of 60 ng±24/10^8 platelets. Plasma HMWK, either in the platelet suspending medium or on the surface of the platelets, could only account for 5% of antigen measured in the solubilized platelets. On the CELISA, platelet HMWK was immunochemically identical to plasma and purified HMWK. In another aspect of this study it was shown that platelet HMWK was secreted from platelets after exposure to ionophore A23187, collagen, and thrombin. Secreted platelet HMWK did not become a part of the platelet Triton-insoluble cytoskeleton. On cross immunoelectrophoresis, secreted platelet total kininogen antigen had a similar electrophoretic migration to plasma total kininogen. Thus, human platelets contain HMWK that can be secreted from platelets and that may participate in plasma coagulation reactions.

Schmaier, A. H., Zuckerberg, A., Silverman, C., Kuchibhotla, J., Tuszynski, G. P., and *Colman, R. W.*

Journal of Clinical Investigation 71:1477-1489, 1983.

Other support: National Heart, Lung, and Blood Institute; Clinical Investigator's Award, Temple University Biomedical Research; American Heart Association; National Institutes of Health, and SCOR.

From the Department of Medicine, Hematology/Oncology Section, and the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

REGIONAL MYOCARDIAL BLOOD FLOW DURING NICOTINE INFUSION AFTER CHRONIC CORONARY ARTERY OCCLUSION: EFFECT OF β-ADRENERGIC BLOCKADE

This study was carried out to evaluate the effect of intravenous nicotine on transmural myocardial blood flow in dogs with chronic occlusion of the left anterior descending coronary artery (LAD). In addition, the effect of the β-adrenergic blocking agent, propranolol, on these changes in transmural myocardial blood flow during nicotine infusion was evaluated. To summarize this work, in eight dogs a portion of the left ventricular free wall (LVFW) was rendered collateral-dependent (CD) by gradual occlusion of the left anterior descending coronary artery with a surgically implanted Ameriod constrictor. Six to eight weeks later, the dogs were anesthetized and regional myocardial blood flow was measured with 7-10 μm radioactive microspheres during (a) control conditions, (b) nicotine alone (24 μg/kg/min i.v.), and (c) nicotine (24 μg/kg/min i.v.) after β-adrenergic blockade with propranolol. During control conditions,

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Nicotine alone increased
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The results indicate (a) tha
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Downey, H. F., Crystal, G

Journal of Cardiovascular

Other support: Southweste

From the Departments of
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PURIFICATION AND PR
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Nathoo, S., Rasums, A., K

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From the Department of Ol
Center, New York.

mean transmural flow was similar in CD, border, and normal regions of the LVFW. Nicotine alone increased flow in all regions of the LVFW with normal (+104%) greater than CD (+56%). These changes in flow were accompanied by increases in mean arterial pressure (+34%) and mean aortic flow (+54%). Nicotine after β-adrenergic blockade appreciably raised mean arterial pressure (+83%) and mean left atrial pressure (+307%), but caused no increase in flow to any region of the LVFW. The results indicate (a) that the nicotine-induced increase in flow is blunted in a CD region, and that (b) β-adrenergic blockade unmasks coronary vasoconstrictor mechanisms during nicotine infusion which prevent increases in flow to normal or CD regions despite increased perfusion pressure and augmented myocardial oxygen demands.

Downey, H. F., Crystal, G. J. and Bashour, F. A.

Journal of Cardiovascular Pharmacology 5(4):685-690, 1983.

Other support: Southwestern Medical Foundation and The Cardiology Fund.

From the Departments of Physiology and Internal Medicine, University of Texas Health Sciences Center, and Cardiovascular Research Laboratory at Methodist Hospital, Dallas.

PURIFICATION AND PROPERTIES OF TWO DIFFERENT α1-PROTEASE INHIBITORS FROM MOUSE PLASMA

Mammalian plasmas contain significant amounts of protein protease inhibitors which serve to regulate several types of extracellular proteolytic activity. In man, these inhibitors have been extensively studied and are well characterized. However, this is not the case for other species. In the murine study presented here, two similar but distinct forms of α1-protease inhibitor (α1-PI) have been isolated and purified 120-fold to homogeneity from the plasma of female, white Swiss mice. The two inhibitors can be separated by chromatography on DEAE-cellulose using a shallow NaCl gradient at pH 8.9 for elution. Because of their differing specificities for elastase and trypsin the two inhibitors have been labeled α1-PI(E) and α1-PI(T), respectively. The apparent M_r for both proteins, as estimated by gel exclusion chromatography, is approximately 53,000 daltons. However by polyacrylamide gel electrophoresis in the presence of SDS, α1-PI(T) has an apparent M_r of 65,000 while the apparent M_r of α1-PI(E) is 55,000. These results suggest differences in charge and carbohydrate composition. The two mouse inhibitors also have different *N*-terminal amino acids. Like human α1-PI, the mouse inhibitors form stable complexes with proteases. However, they differed from human α1-PI in that they were not found to neutralize either human thrombin or plasmin. While α1-PI(E) inhibits bovine pancreatic trypsin, chymotrypsin, and porcine pancreatic elastase, α1-PI(T) is an effective inhibitor only of trypsin. Plasma levels of α1-PI(E) increase significantly 24 hrs. after stimulation of the acute phase reaction while those of α1-PI(T) do not. These data, which suggest that α1-PI(E) and α1-PI(T) are products of different genes, may help interpret the results of experiments in which the mouse has been used as an animal model for human lung disease.

Nathoo, S., Rasums, A., Katz, J., Ferguson, W. S., and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 219(2):306-315, 1982.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

FORMATION AND STABILITY OF THE COMPLEX FORMED BETWEEN HUMAN ANTITHROMBIN-III AND THROMBIN

Antithrombin III (AT-III) is a glycoprotein found in mammalian plasma that inhibits thrombin and certain other serine proteases. In an earlier set of experiments, these investigators found that the inhibition of thrombin by antithrombin-III proceeds by formation of a covalent bond between the two molecules, a bond that is resistant to both reduction and to denaturation by sodium dodecyl sulfate (SDS). The paper presented here shows further that the inhibition of thrombin by antithrombin-III involves formation of a 1:1 covalent complex between protease and inhibitor and concomitant cleavage of the antithrombin-III peptide chain after Arg-385. The resultant fragment remains connected to the complex via a disulfide bond. This complex spontaneously breaks down into a fragment of approximately 55,000 daltons and smaller peptides. Breakdown is prevented by the presence of hydroxylamine or diisopropylflurophosphate, or by denaturation with urea. It occurs even if the purified complex is treated with diisopropylflurophosphate prior to purification, and can be greatly accelerated by the presence of small amounts of active thrombin. The initial sites of proteolytic attack on the complex are after Arg-13 of the thrombin A chain and Arg-68 of the thrombin B chain. These data indicate that active thrombin can be released from the antithrombin-thrombin complex and that thrombin becomes more susceptible to proteolytic attack when complexed with antithrombin.

Ferguson, W. S. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 220(1):301-308, 1983.

Other support: New York Heart Association.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

LOCALIZATION OF THE DISULFIDE BOND IN HUMAN ANTITHROMBIN III REQUIRED FOR HEPARIN-ACCELERATED THROMBIN INACTIVATION

Antithrombin III (AT-III) contains three disulfide bonds and no free sulfhydryl groups. It was previously reported that only one of the AT-III disulfide bonds is reduced in the absence of denaturing agents. In this study, it is demonstrated that heparin accelerates the rate of inhibition of thrombin by antithrombin III. Reduction of one of the three antithrombin disulfide bonds with dithiothreitol under mild conditions abolishes this rate-enhancing effect without affecting the rate of reaction in the absence of heparin. Alkylation of mildly reduced antithrombin III with [^{3}H]iodacetic acid followed by digestion with cyanogen bromide yielded two major labeled peptides. The smaller peptide, containing Cys-422, was identified as extending from Gly-414 to the C-terminus, Lys-424. These data are consistent with the larger labeled peptide being the one extending from Glu-104 to Met-243 and containing Cys-239. Cys-422 has been shown by other investigators to be linked to Cys-239. These data indicate that the sensitive disulfide bond in antithrombin III extends between Cys-239 and Cys-422; the site at which thrombin cleaves the antithrombin III is between these two half-cystines.

Ferguson, W. S. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 221(1):304-307, 1983.

Other support: New York Heart Association.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

CIGARETTE SMOKING LIPOPROTEINS

This investigation foc
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Mulligan, J. J., Cluette, J.

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Other support: American H

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ANGIOTENSION-CONV ASSAY

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Ryan, J. W., Chung, A. a

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Other support: U. S. Publ

From Department of Medic

CIGARETTE SMOKING IMPAIRS HEPATIC UPTAKE OF HIGH DENSITY LIPOPROTEINS

This investigation focused on tobacco smoke-induced alterations in hepatic uptake and metabolism of high density lipoprotein (HDL). Specifically, the effect of chronic inhalation of cigarette smoke on hepatic uptake of HDL in White Carneau pigeons was examined. Four treatment groups included: (1) Shelf control birds fed a chow diet and retained in their cages; (2) Sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by a smoking machine; (3) Low nicotine-low carbon-monoxide (LoLo) animals also fed the cholesterol diet and exposed to low concentrations of these cigarette smoke products; and (4) High nicotine-high carbon monoxide (HiHi) birds fed the cholesterol diet and subjected to high concentrations of these components. Livers from both smoke-exposed groups contained significantly more triglyceride than those from Sham animals while livers from HiHi birds alone had elevated concentrations of protein. Liver slices from LoLo and HiHi pigeons incorporated significantly less HDL ^{3}H free and esterified cholesterol and HDL ^{14}C apoprotein from media during *in vitro* incubation than livers from Sham birds. The results from this study are unique in that they provide the first evidence for a mechanism by which cigarette smoking may impair delivery of HDL to hepatic tissue and thus potentially attenuate anti-atherogenic properties of this lipoprotein.

Mulligan, J. J., Cluette, J. E., Kew, R. R., Stack, D. J., and *Hojnacki, J. L.*

Biochemical and Biophysical Research Communications 112(3):843-850, 1983.

Other support: American Heart Association, Greater Boston Massachusetts Division.

From the Biochemistry Program, Department of Biological Sciences, University of Lowell, Lowell, MA.

ANGIOTENSION-CONVERTING ENZYME: I. NEW STRATEGIES FOR ASSAY

Although the disposition of converting enzyme (kininase II) on the luminal surface of pulmonary endothelial cells is well established and it is recognized that there is a net conversion of angiotensin I into angiotensin II as blood passes through the lungs, little is known at this time about modulations of converting enzyme activity that may arise through, *e.g.*, changes in the quality of inhalants, blood flow or blood oxygenation. In the present effort to develop simpler and more precise means of measuring angiotensin-converting enzyme, a series of acylated tripeptides, each bearing a radioisotope in the moiety used for acylation, was synthesized. Results showed that the enzyme, a dipeptidyl carboxypeptidase, is capable of removing C-terminal dipeptides from a variety of oligopeptides. The following compounds were synthesized here: *p*-I-benzoyl-Gly-Gly-Gly (I), *p*-I-benzoyl-Pro-Phe-Arg (II), *p*-I-benzoyl-Gly-His-Leu (III), *p*-I-benzoyl-Phe-Ala-Pro (IV), *p*-I-benzoyl-Phe-His-Leu (V), and *p*-I-benzoyl-Phe-Ser-Pro (VI). Each of the compounds can be labelled by dehalogenation in $^{3}H_2$ gas. These substrates can be used *in vitro* or *in vivo* to measure converting enzyme. The choice of substrate depends on the goals of the experiment: substrate I or III when wide variations in substrate concentrations are needed, but high sensitivity is not; substrate IV when high sensitivity is needed.

Ryan, J. W., Chung, A. and *Ryan, U. S.*

Environmental Health Perspectives 35:165-170, 1980.

Other support: U. S. Public Health Service and the Hartford Foundation.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

SIMPLE RADIOASSAYS FOR HUMAN PLASMA AND GLANDULAR KALLIKREINS

Five years ago, a program to develop a specific and sensitive assay for the kallikreins was begun. Through this program, Pro-Phe-Arg-[^{3}H]benzylamide and Pro-Phe-Arg-[^{3}H]anilide (and the corresponding [^{14}C]anilide) were synthesized and developed. Both substrates proved to be highly reactive with human glandular kallikreins, but neither proved to be very reactive with human plasma kallikrein. To obtain a radiolabeled substrate for plasma kallikrein, (D)Pro-Phe-Arg-[^{3}H]benzylamide, a compound modeled after the potent plasma kallikrein alkylating agent, (D)Pro-Phe-Arg-chlormethylketone was prepared. This substrate proved to be even less reactive with human plasma kallikrein than was Pro-Phe-Arg-[^{3}H]benzylamide. Surprisingly, though, it proved to be more reactive with human glandular kallikrein than was the (L)Pro-analog. After this, a search was begun for amino acid residues (and related acyl groups) that might be used to replace Pro or (D)Pro in order to enhance the affinity of substrate for plasma kallikrein. Of twelve acyl groups thus surveyed, <Glu(5-keto-L-Pro) proved to be the best. <Glu-Phe-Arg-[^{3}H]benzylamide is highly reactive with plasma kallikrein. These results indicate that subtle changes in the side-chain of the P_3 subsite of tripeptide substrates for the kallikreins can convey profound changes in selectivity and kinetic behavior. In summary, this paper contains the routine assay protocols for human urinary kallikrein (HUK) and human plasma kallikrein (HPK). The data obtained following these protocols indicate the HUK and HPK have somewhat different requirements in terms of the side-chains of their tripeptide-benzylamide substrates, and there are differences in terms of the substrates themselves.

Ryan, J. W. *et al. (Ryan, U. S.)*

Advances in Experimental Medicine and Biology 156A:241-249, 1983.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

BRADYKININ-INDUCED RELEASE OF PROSTACYCLIN AND THROMBOXANES FROM BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS: STUDIES WITH LOWER HOMOLOGS AND CALCIUM ANTAGONISTS

The purpose of this study was to investigate the ability of bradykinin to release prostaglandin I_2 and thromboxane A_2 from endothelial cells derived from bovine pulmonary arteries. The results presented in this paper show that bovine pulmonary artery endothelial cells, in serum-free culture medium, release small quantities of prostacyclin and thromboxane A_2 (3-10 and 0.1-0.3 ng/ml; measured as immunoreactive 6-ketoprostaglandin $F_1\alpha$ and thromboxane B_2, respectively). The release of these substances is stimulated by up to 20-fold during a 3 min incubation with the vasodilator, bradykinin. Endothelial cells incubated with [^{3}H]arachidonic acid for 24 h and then exposed to bradykinin for 3 min release ^{3}H into the medium, approximately 65% of which co-chromatographs with 6-ketoprostaglandin $F_1\alpha$ and 3% with thromboxane B_2. The effects of bradykinin are dose-related and are often discernible when the hormone is used at concentrations believed to occur physiologically (10 pg/ml). Furthermore, the bradykinin molecule must be intact; none of its lower homologs affects the release

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Other support: U. S. F

From the Research Divi
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ASSAY OF ANGIOTE

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Other support: U. S. Pu

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of prostacyclin, thromboxane A_2, or ^3H unless used at concentrations (1 μM or higher) unlikely to be achieved *in vivo*. The release appears to involve calcium uptake and calmodulin: it is abolished by EGTA and inhibited by the "slow channel" calcium antagonists, verapamil and nifedipine, and by the calmodulin inhibitor, trifluoperazine. These findings suggest that bradykinin exerts some of its hormonal effects by acting on specific receptors possessed by vascular endothelial cells; receptor activation is associated with calcium transport, arachidonate mobilization, and a selective synthesis of prostacyclin, a vasodilator in its own right.

Crutchley, D. J., Ryan, J. W., *Ryan, U. S.*, and Fisher, G. H.

Biochimica et Biophysica Acta 751:99-107, 1983.

Other support: U. S. Public Health Service.

From the Research Division, Miami Heart Institute, Miami Beach, FL and the University of Miami School of Medicine, Miami, FL.

ASSAY OF ANGIOTENSIN CONVERTING ENZYME *IN VIVO*

Angiotensin converting enzyme (ACE) is known to be capable of hydrolyzing acylated tripeptides, and a standard assay system for ACE has been used for several years now. In the work reported here, however, a series of acylated tripeptides was prepared to bear a radioactive label in their organophilic moieties. Thus, [^3H]hippuryl-His-Leu and [^3H]benzoyl-Phe-Ala-Pro were prepared; the latter being an analog of BPP_{5a}, an ACE substrate of exceptional affinity. Recently, the use of these substrates has been recommended for the assay of ACE *in vivo*. The protocols for these assays are presented in this paper. Results show that the hydrolysis of [^3H]benzoyl-Phe-Ala-Pro during a single passage through the lungs is far more extensive than that of [^3H]hippuryl-His-Leu. For many experimental purposes, a control hydrolysis rate of about 60% provides a useful baseline for examining for variables that may affect the rate of hydrolysis. The hydrolysis of benzoyl-Phe-Ala-Pro *in vivo* is not inhibited by an inhibitor of aminopeptidase A, α-L-Glu-CH_2Br, nor by an inhibitor of carboxypeptidase N, 2-mercaptomethyl-3-guanidinoethylthiopropionic acid. The *in vivo* assays described here are by no means convenient for routine clinical use. However, the assays may well be used in phase I clinical trials and in critically ill patients whose blood pressures are difficult to control. On the basis of the work discussed here, it can be said that given the appropriate cannulation, enzyme activity of any vascular bed can be examined, including those used commonly (*e.g.*, hamster cheek pouch) to study the physiology of the microcirculation. Overall, it is believed that this approach to the assay of selected enzymes *in vivo* has wide applicability and may provide means of improving current understanding of the physical regulation of enzyme activities of living animals.

Ryan, J. W., Berryer, P. and Chung, A. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:805-812, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

ANALOGS OF BRADYKININ CONTAINING DEHYDROPHENYLALANINE

Analogs of bradykinin (BK) containing α, β-dehydrophenylalanine (ΔPhe) in place of L-phenylalanine in positions 5 or 8, or 5 and 8 were synthesized and tested in this laboratory recently. All three of these analogs proved to be markedly more resistant than BK to degradation during passage through the pulmonary vascular bed. On the basis of results seen here, it was postulated that ΔPhe, especially when substituted in position 5, conferred resistance to degradation by kininase enzymes. In order to pursue this speculation further, ΔPhe^5-BK, ΔPhe^8-BK, and ΔPhe^{5,8}-BK were examined for their apparent affinities for angiotensin converting enzyme (ACE) (also known as kininase II, the major kininase enzyme of the lungs). Results show that, on the basis of relative I_{50} values, BK itself clearly has a greater affinity for ACE than do the ΔPhe-containing analogs of BK (BK>ΔPhe^8-BK > ΔPhe^5-BK>ΔPhe^{5,8}-BK). Comparable examinations were made for the influence of ACE *in vivo*. In conclusion, on the basis of these and other studies, it is evident that the biologic effects of BK are heavily influenced by kininase enzymes, the most important of which is ACE. BK analogs of high apparent potency, *e.g.*, ΔPhe^5-BK, may owe much of their apparent potency to resistance to enzymic degradation as well as to effective hormone-receptor interactions. Finally, it seems more and more apparent that the interactions of BK and other substrates with ACE can be heavily influenced by rather subtle changes in side-chains or amino acid residues distant from the catalytic site of the enzyme-substrate complex.

Fisher, G. H., Ryan, J. W. and Beryer, P. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:607-612, 1983.

Other support: U. S. Public Health Service, American Lung Association, and American Heart Association, Florida Affiliate.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

EFFECTS OF BRADYKININ AND ITS HOMOLOGS ON THE METABOLISM OF ARACHIDONATE BY ENDOTHELIAL CELLS

Bradykinin can stimulate cellular prostaglandin (PG) biosynthesis. Earlier studies have shown that high doses of BK release PGs from isolated perfused lungs and low doses of BK release PGs from cultured endothelial cells. This study was started to find out whether BK has the ability to release prostacyclin (PGI_2) and other metabolites of arachidonic acid (AA) from cultured pulmonary arterial endothelial cells. BK was also compared here with seven of its lower homologs, including two products formed by angiotensin I converting enzyme (ACE): Arg^1-Phe^5 and Arg^1-Pro^7. Results from thin-layer chromatographic analysis showed that most of the AA released by BK was metabolized to PGI_2, with lesser amounts of $PGF_{2\alpha}$ and TXA_2 being formed. Comparatively, BK was by far the most potent of the peptides studied, the most active homolog, Des-Arg^1-BK, possessing less than 1% of the activity of BK. In summary, the results presented in this paper show that low doses of BK can induce a rapid release of PGI_2 from cultured pulmonary arterial endothelial cells, a finding consistent with the hypothesis that some of the effects of BK on vascular tone may be mediated via release of PGI_2. These results reemphasize the importance of BK-metabolizing enzymes such as ACE, since all of the lower homologs tested had less than 1% of the activity of BK.

Crutchley, D. J., Ryan, J. W., *Ryan, U. S.*, Fisher, G. H., and Paul, S. M.

Advances in Experiment

Other support: U. S. Pu

From the Research Divisi
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Fisher, G. W. and Ryan,

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From the Department of M

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Advances in Experimental Medicine and Biology 156A:527-532, 1983.

Other support: U. S. Public Health Service.

From the Research Division, Miami Heart Institute, Miami Beach, FL, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

DISTANT BINDING SITES OF KININASE II

In this attempt to examine the complexities of the binding sites of kininase II, ongoing studies of the structure-activity relationships of BPP_{9a}, a potent inhibitor of kininase II, have been expanded to include all of the possible lower homologs and selected analogs of BPP_{9a}. For this study the lower homologs and analogs of BPP_{9a} were synthesized by the solid-phase technique, C-terminal amides and C-terminal esters were synthesized in various ways, the final peptides were examined for purity, and inhibitory potencies were measured. Results showed, among other things, that five of the lower homologs are as potent as BPP_{9a} itself. The nature of the last three amino acid residues comprising the C-terminus appears to be important for significant interaction of the peptide with the active site of the enzyme. Although it was previously believed that a free C-terminal carboxyl group was necessary for inhibitory activity, it was found here that there are peptides, each with a blocked C-terminus (ester or amide), which retain high kininase II inhibitory activities. Among the many other observations considered in this paper is that tryptophan at position two of BPP_{9a} is critical for binding of the peptide to the enzyme. However, tryptophan can be replaced in BPP_{9a} by other aromatic acids, such as phenylalanine or tyrosine, with retention of inhibitory activity. Other things noted include: (1) the length of the side chain group at residue six does not appear to be critical. (2) Size *per se* of a peptide does not appear to be a critical factor in terms of the affinity of binding. (3) Certain peptide sequences have been found in which a free C-terminal carboxyl group is not necessary for binding; for example, the C-terminal amides or esters of the (1-9), (2-9), (1-7), and (2-7) lower homologs are potent inhibitors. All of these sequences contain tryptophan. These data, therefore, suggest the importance of additional binding site(s) at a distance from the catalytic site of the enzyme, one filled well by an aromatic amino acid such as tryptophan. The requirements for binding to the distant site(s) appear to differ in some respects from those for the proximal sites, and (4) A positively-charged residue at the active site of the enzyme is thought to bind to the negatively-charged C-terminal carboxyl group of BPP_{9a}. Binding of the free α-carboxyl group of the C-terminal residue may be the first step to enzyme-inhibitor interaction but may not be an absolute requirement for binding. Overall, it seems, within limits, that these investigators have begun to strengthen the hypothesis that compounds can be made which occupy the distant binding site(s) but not those at or near the catalytic site. Presumably bradykinin, a nonapeptide, and angiotensin I, a decapeptide, require access to the distant binding site(s) as well as the proximal site.

Fisher, G. W. and Ryan, J. W. (*Ryan, U. S.*)

In: Fritz, H., Back, N., Dietze, G., and Haberland, G. L. (eds.): *Kinins-III Pt. B*, New York: Plenum Publishing Corporation, 1983, pp. 813-821.

Other support: U. S. Public Health Service and American Heart Association, Florida Affiliate.

From the Department of Medicine, University of Miami, Miami, FL.

A PEPTIDE FORMED BY FIBRINOLYSIS INCREASES MICROVASCULAR PERMEABILITY AND INHIBITS ANGIOTENSIN CONVERTING ENZYME

It is shown in this paper that a fibrin(ogen) pentapeptide, Ala-Arg-Pro-Ala-Lys, increases microvascular permeability and is an inhibitor of angiotensin converting enzyme. This peptide and a variety of its synthetic analogs and homologs were assayed for their abilities to inhibit angiotensin converting enzyme *in vitro*, and the resulting data were compared to the abilities of these compounds to increase microvascular permeability. Although the parent peptide and many of its analogs and homologs were moderately potent inhibitors of the enzyme, no clear relation was established between enzyme inhibition and direct effects of the peptides on microvascular permeability.

Saldeen, T., Ryan, J. W. and Ragnarsson, U. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:829-834, 1983.

Other support: U. S. Public Health Service and the Swedish Medical Research Council.

From the Institutes of Forensic Medicine and Biochemistry, University of Uppsala, Uppsala, Sweden, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ASSAYS OF COMPONENTS OF THE KALLIKREIN-KININ SYSTEM BASED ON FIRST ORDER REACTION KINETICS

It is widely recommended that enzyme activities be measured *in vitro* under conditions approximating zero order reactions although reactions like these are unlikely to occur *in vivo*. Indeed, *in vivo* the reactions between, *e.g.*, bradykinin and angiotensin converting enzyme probably occur in such a way that the substrate concentration, [S], is well-below Km([S]<-Km). Often conditions for true first order reaction kinetics cannot be obtained because of inability to detect and measure substrate utilization and/or product formation. This is especially true for chromogenic substrates capable of yielding a weak chromophore. However, the problem of detecting substrate depletion or product formation is largely overcome when the substrate possesses a radioactive leaving group readily separated from the substrate itself. In the present study, such substrates were used to examine empirically for advantages and disadvantages of measuring enzyme activities *in vitro* under conditions in which So <<Km; conditions similar to those likely to exist *in vivo*. Among the concluding comments of this paper, it is noted that radiolabeled substrates of high specific radioactivity can be used (without carrier) at concentrations well within the range of first order kinetics. The latter use has many advantages, one of which is that radiolabeled substrates, in extremely low concentrations, can be used to measure certain components of the kallikrein-kinin system *in vivo* under conditions that simulate those for, *e.g.*, the formation and inactivation of bradykinin. However, the principles of these assays are general and are by no means limited to the kallikrein-kinin system.

Carlin, G., Ryan, J. W. and Saldeen, T. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:797-804, 1983.

Other support: U. S. Public Health Service.

From the Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden, and Department of Medicine, University of Miami School of Medicine, Miami, FL.

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POTENTIATION OF BRADYKININ BY INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME

Bradykinin (BK) has a higher affinity for angiotensin converting enzyme (ACE) than does angiotensin I (AI) and, as the better substrate, should compete more effectively with a competitive inhibitor of ACE, and should be more difficult to preserve. However, empirically, inhibitors of ACE are effective as BK potentiating agents at concentrations too low to inhibit discernibly the rate of conversion of AI into angiotensin II (AII). In the present study, the investigators have examined this apparent paradox experimentally and on theoretical grounds. Results of this study have shown that the more efficiently BK is inactivated under control conditions, the more readily its effects can be potentiated by an ACE inhibitor. To be precise, in this study female Sprague-Dawley rats were anesthetized with i.p. pentobarbital and cannulas were placed into a femoral vein for i.v. injections and into a femoral artery for direct recording of blood pressure (BP). Each rat was then tested for its responsiveness (BP effects) to intravenously administered angiotensin II, angiotensin I, and bradykinin. Log dose-response curves were constructed and an angiotensin converting enzyme inhibitor (usually benzoyl-Phe-3-thio-D-isobutyrl-L-Pro, BPTIP) was given intravenously in such a small dose that the BP response to AI would recover to control values over the course of about 45-60 minutes. Following injection of the inhibitor, alternate injections of BK and AI were made. Results showed that as little as 10 ng of BK caused a discernible fall of BP. Although AI did have a measurable effect on BP, overall it is clear that the effects of BK on BP are potentiated well beyond the time of inhibition of ACE as judged by recovery of the BP response to AI.

Ryan, J. W., Carlin, G., Berryer, P., and Chung, A. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:613-620, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden.

KININS, ENDOTHELIAL CELLS AND CALMODULIN

Since calmodulin, a rather ubiquitous substance, is believed to participate in a vast array of intracellular activities, the present studies were begun to investigate two major questions about this substance: (1) Do bovine pulmonary artery endothelial cells, cells known to be responsive to bradykinin (BK), contain calmodulin? (2) If the cells contain calmodulin, is their responsiveness modulated by pretreatment of the cells with an agent reputed to compete with Ca^{++} for calmodulin binding sites? In the search for these answers, the endothelial cells were examined by immunocytochemical techniques for the subcellular disposition of calmodulin. (Given the large number of fundamental cellular activities believed to be controlled in whole or part by calmodulin, surprisingly little is known of its distribution within cells.) Because endothelial cells grow in monolayer, it was believed that the cells in culture provide an unusually favorable system for localizing calmodulin at the ultrastructural level. Overall, the data presented in this paper show that endothelial cells, like all other eukaryotic cells yet examined, contain calmodulin. The distribution of calmodulin within the cells is

characteristic and well defined. Although these studies have just begun, it is not unreasonable to suggest that endothelial cells in culture may be extraordinarily amenable to efforts to define the functions of calmodulin in terms of cell structure. Firstly, the cells grow in monolayer throughout their life span. Secondly, the cells *in vivo* are exposed to all hormones and any drugs that may be administered systemically to the host organism. Thirdly, the cells are responsive to hormones, *e.g.*, BK, known to affect vascular tone, and at least one of the responses to BK (release of PGI_2) is inhibited by trifluoperazine; and fourthly, the intact cells, as opposed to disrupted tissue preparations, can be used for concurrent biochemical and morphologic studies.

Ryan, U. S. and Ryan, J. W.

In: Fritz, H., Back, N., Dietze, G., and Haberland, G. L. (eds.): *Kinins-III Pt. A*, New York: Plenum Publishing Corporation, 1983, pp. 671-679.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

INHIBITION OF THE FORMATION OF ANGIOTENSIN III

In this attempt to improve understanding of the immediate metabolic fate of angiotensin II (and the means by which angiotensin II may be converted into des-Asp^1-angiotensin II; also known as angiotensin III), a series of inhibitors and a radiolabeled substrate for aminopeptidase A have been prepared. Angiotensin II, given intravenously, is known to be more potent as a blood pressure raising agent than is angiotensin III, and it has been found that the inhibitors, a-L-aspartic acid chloromethylketone and a-L-glutamic acid bromomethylketone, can potentiate the blood pressure effects of angiotensin II. Conversely, angiotensin III has been reported by others to be more potent than angiotensin II as an aldosterone secretogogue *in vitro*. However, it seems at this point that it should be feasible to use the inhibitors prepared here to distinguish the aldosterone secretogogue effects of angiotensin II and angiotensin III *in vivo*. In this study, the effects of a-L-Asp-CH_2Cl on the aminopeptidase A and on arginine aminopeptidase activities of rat lung homogenates were noted. As shown here, the arginine aminopeptidase, unlike aminopeptidase A, does not require added Ca^{++}. Results of a companion study show the effects of a-L-Asp-CH_2Br, given in a bolus injection of 2 mg in 50 μL of saline, on the intrinsic activity of plasma aminopeptidase A. These results, expressed in terms of first order enzyme units, provide evidence to indicate that the drug, given i.v., effectively inhibits aminopeptidase A *in vivo*. Also, the effects of a-L-Glu-CH_2Br, given by i.v. infusion, on the blood pressure response to angiotensin II are shown. The results presented here show that apparently specific inhibitors of aminopeptidase A are capable of potentiating the effects of angiotensin II given intravenously. For future research purposes, it should be noted from this work that aminopeptidase A inhibitors, unlike angiotensin converting enzyme inhibitors, affect the renin-angiotensin system but not the kallikrein-kinin system.

Chung, A., Ryan, J. W. and Berryer, P. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:693-701, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

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METABOLISM OF BRADYKININ BY ENDOTHELIAL CELLS IN CULTURE

Since inhibitors of angiotensin converting enzyme (ACE) are coming into common clinical use, it is becoming increasingly important to define the metabolic fate(s) of bradykinin (BK) under conditions in which ACE is completely or largely inhibited. Thus, the present study was begun with two goals in mind: (1) to confirm that endothelial cells possess on their surfaces a multiplicity of enzymes capable of inactivating BK and (2) to examine which of these enzymes are functionally significant "kininase" enzymes and remain active in the presence of an ACE inhibitor. The first thing studied for this was the metabolism of [^{3}H]Phe^8-BK by isolated, perfused rat lungs. After suitable preparation, radioactive fractions were collected and then examined by paper electrophoresis at pH 2 and pH 5 and by thin layer chromatography. Authentic standard lower homologs of BK were included in the analysis for comparative purposes. These isolated lung perfusion studies were performed in order to provide a standard of comparison with the metabolism of [^{3}H]Phe^8-BK by endothelial cells in culture. In the second series of experiments, post-confluent cultures of bovine pulmonary endothelial cells were used. Within the limits of these studies, it appears that bovine pulmonary artery endothelial cells in culture metabolize BK to yield products like those formed by isolated perfused rat lungs. In a third series of experiments, the metabolism of [^{3}H]Pro^7-BK by endothelial cells in culture was examined. As expected, the major radioactive metabolite (82% of total radioactivity in medium after 120 min of incubation) was Ser-Pro, the dipeptide formed by ACE from Arg^1–Pro^7, a product of the reaction of ACE with BK. As implied by the results of the above-described experiments, isolated rat lungs and bovine pulmonary endothelial cells do not degrade BK through the action of a carboxypeptidase B-like enzyme. At present, it remains unclear which of the endothelial cell enzymes, other than ACE itself, act as true "kininase" enzymes and which merely act to degrade further lower homologs of BK formed by enzymes of primary importance. Clearly, only those enzymes that degrade BK itself as it occurs physiologically merit the term "kininase."

Ryan, J. W., *Ryan, U. S.*, Chung, A., and Fisher, G. H.

Advances in Experimental Medicine and Biology 156B:775-781, 1983.

Other support: U. S. Public Health Service and the American Lung Association.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

THIOL-DISULFIDE-DEPENDENT INTERCONVERSION OF ACTIVE AND LATENT FORMS OF RAT HEPATIC 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE

The significance of thiols in liver cholesterol biosynthesis, particularly in the rate-controlling enzyme hydroxymethylglutaryl-CoA reductase, was investigated here. The studies showed that the hepatic hydroxymethylglutaryl-CoA reductase exists in two forms: active (E_a) and inactive latent (E_i) forms. The interconversion between the two forms of the enzyme is thiol-mediated and the maximal degree of activation of E_i depends on the activating thiol with the order of effectiveness: dithioerythritol = dithiothreitol>glutathione (GSH)>cysteine. E_a is inhibited by oxidized glutathione (GSSG). The degree of the inhibition of E_a by GSSG is proportional to the ratio GSSG/thiol in the reaction. E_i was solubilized from microsomes and purified. Its molecular

weight is estimated to be 104,000 by gel filtration chromatography on Sepharose 6B. The reducing agents $NaBH_4$, dithionite and ascorbate failed to activate E_i. $NaBH_4$ did not inhibit E_a whereas only partial inhibition was caused by ascorbate and dithionite. Soluble E_i binds to both blue dextran/Sepharose 4B and agarose/hexane-3-hydroxy-3-methylglutaryl Coenzyme A affinity resins at low-salt concentrations. By contrast, soluble E_i did not bind to agarose/hexane-hydroxymethylglutaryl-CoA whereas quantitative binding of E_i to blue dextran/Sepharose 4B was still observed at low salt concentrations. These results indicate that thiols are necessary cofactors for hydroxymethylglutaryl-CoA reductase reactions. Their effect on the activation of E_i is not caused by change in the state of aggregation of the enzyme. Rather, the reversible change of the enzyme from E_i to E_a is affected by increasing the affinity of the enzyme to the substrate hydroxymethylglutaryl-CoA.

Dotan, I. and *Shechter, I.*

Biochimica et Biophysica Acta 713:427-434, 1982.

From the Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

TISSUE SITES OF DEGRADATION OF APOPROTEIN A-I IN THE RAT

In this paper, apoprotein A-I was labeled with a radiolabeled ligand that remains trapped intracellularly following uptake and degradation of the protein moiety. Specifically, the tissue sites of degradation of apoprotein A-I were determined in the rat *in vivo* using a newly developed tracer of protein catabolism, an adduct of ^{125}I-tyramine and cellobiose, which is then covalently coupled to the protein. This methodology takes advantage of the fact that when a protein labeled with ^{125}I-tyramine-cellobiose is taken up and degraded, the radiolabeled ligand remains trapped intracellularly. Thus radio-iodine accumulation in a tissue acts as a cumulative measure of protein degradation in that tissue. In this instance, apoprotein A-I (apo-A-I) was labeled with tyramine-cellobiose (TC) and the TC-labeled apo-A-I was then reassociated with high density lipoprotein (HDL) *in vivo* by injection into donor animals. After 30 min., serum from donor animals was recovered and injected into recipient rats. TC-labeled apo-A-I in the donor serum was shown to be exclusively associated with HDL. The fractional catabolic rate of ^{125}I-TC-apo-A-I was not significantly different from that of conventionally labeled apo-A-I. The kidney was the major site of degradation, accounting for 39% of the total. The liver was responsible for 26% of apo-A-I catabolism, 96% of which occurred in hepatocytes. The kidney was also the most active organ of catabolism/g of wet weight. The tissues next most active/g of wet weight were ovary and adrenal, a finding that is compatible with a special role of HDL in the rat for delivery of cholesterol for steroidogenesis. Preliminary studies using HDL labeled both with ^{125}I-TC-apo-A-I and [^{3}H]cholesterol ethers again demonstrated high rates of renal uptake of apo-A-I but less than 1% of total ether uptake. It is postulated that the high activity of kidney was not due to uptake of intact HDL particles, but rather to glomerular filtration and tubular reabsorption of free apo-A-I.

Glass, C. K., Pittman, R. C., Keller, A., and *Steinberg. D.*

The Journal of Biological Chemistry 258(11):7161-7167, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

LIPOPROTEINS AN
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G. Lyman Duff
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Steinberg, D.

Arteriosclerosis 3:293-3

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LIPOPROTEINS AND ATHEROSCLEROSIS: A LOOK BACK AND A LOOK AHEAD

G. Lyman Duff was one of the greats among the pioneers working with the complex problem of atherosclerosis. In this Duff Memorial Lecture, the presenter attempts to approach the atherogenic process through the advances in biochemistry and cell biology which have given researchers the concepts and the techniques with which to frame relevant questions about atherosclerosis. There are three specific hypotheses that are being actively considered as of now–The Lipid Hypothesis, the Endothelial Injury Hypothesis, and the Unified Hypothesis. In defense of the first hypothesis, it is pointed out that lipoproteins, most specifically low density lipoproteins (LDL), can be a sufficient cause of atherosclerosis. This is shown most strongly by the genetic evidence of patients with familial hypercholesterolemia, and the genetic argument has recently been strengthened by the development in Japan of a unique strain of LDL-receptor-deficient rabbits, the Watanabe or WHHL strain. Turning to the other major "school" of atherogenesis, the Endothelial Injury Hypothesis, it is noted that endothelial damage–possibly subtle–causes blood platelets to adhere and aggregate. This causes release of the platelet-derived growth factor (PDGF), discovered by Ross and his coworkers, that stimulates smooth muscle cells to proliferate and to secrete connective tissue matrix elements. Macrophages and endothelial cells have been shown recently to secrete growth factors. Thus, repeated episodes of endothelial damage and repeated bouts of smooth muscle cell replication can lead to the development of the "space-occupying lesion" that is recognized as an atheroma. From the presenter's point of view, these two views of atherogenesis, far from representing two contesting "schools" in the classical sense, are better regarded as simply two faces of a unified Janus hypothesis. There are at least six interactions between these two hypotheses that make them almost inseparable, as can be seen in Figure 4 in this paper. In view of these many potential interactions, some firmly established, others needing additional elaboration, it seems that some version of this middleground Unified Hypothesis should become acceptable to most workers in the atherosclerosis research field. The remainder of this closely considered paper is devoted primarily to LDL and how it may promote atherogenesis.

Steinberg, D.

Arteriosclerosis 3:293-301, 1983.

Other support: National Heart, Lung and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

DISSOCIATION OF TISSUE UPTAKE OF CHOLESTEROL ESTER FROM THAT OF APOPROTEIN A-I OF RAT PLASMA HIGH DENSITY LIPOPROTEIN: SELECTIVE DELIVERY OF CHOLESTEROL ESTER TO LIVER, ADRENAL, AND GONAD

Since there has been increasingly impressive evidence that risk of coronary heart disease correlates inversely with plasma high density lipoprotein (HDL) levels, much research has been stimulated on HDL metabolism and the underpinnings of the noted

protective effect. In the work presented here, the metabolic fate of homologous HDL was studied in the rat, tracing the apoprotein A-I (apo A-I) and cholesterol ester moieties simultaneously. The apo A-I was labeled with covalently linked ^{125}I-labeled tyramine cellobiose, which accumulates in the cells degrading the apoprotein; [^{3}H]cholesterol ethers, which cannot be hydrolyzed or mobilized after uptake, were incorporated into the lipid core of reconstituted HDL to reflect the fate of the cholesterol esters. Several lines of evidence, including direct comparison with biologically labeled HDL, are presented to support the validity of this approach. The liver was the major organ of cholesterol ether uptake, accounting for 65% of the total; the adrenal gland and ovary were the most active organs per gram (wet) weight. Uptake of cholesterol ether was 7-fold greater than that of apo A-I in adrenal, 4-fold greater in the ovary, and >2-fold greater in the liver. The remaining tissues took up apo A-I and cholesterol ethers at more nearly equal rates. Transfer of HDL-associated cholesterol ethers and ^{125}I-labeled apo A-I to other lipoprotein fractions was not observed; thus, the results reflect direct uptake from HDL itself. Whereas uptake of low density lipoprotein appears to involve endocytosis of intact particles, uptake of HDL in at least some rat tissues involves additional, more complex, transfer mechanisms.

Glass, C., Pittman, R. C., Weinstein, D. B., and *Steinberg, D.*

Proceedings of the National Academy of Sciences of the United States of America 80:5435-5439, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

ASSOCIATION OF COAGULATION FACTOR V WITH THE PLATELET CYTOSKELETON

In order to investigate a possible interaction of the platelet cytoskeleton with plasma membrane components, these studies focused on two coagulation proteins that have been shown to bind to high affinity receptor sites on the platelet surface: Factor V and Factor Xa. Immunological and functional evidence is presented to show that these receptor sites are also associated with the platelet cytoskeleton. Specifically, triton-insoluble cytoskeletons from thrombin-activated platelets have been shown to contain Factor Va. This conclusion is based on the following evidence (a) a monoclonal antibody to Factor V inhibits the cytoskeleton's ability to potentiate the Factor Xa-catalyzed activation of prothrombin and the ability of cytoskeletons to correct the clotting defect of Factor-V-deficient plasma; (b) the properties of cytoskeletal-associated Factor Va and purified Factor Va are similar; for example, both factors are inhibited by EDTA and heat but not by proteolytic enzyme inhibitors such as DFP; (c) cytoskeletal Factor V is most likely in the activated form, Factor Va, since further treatment of the washed cytoskeletons with thrombin does not increase its Factor V coagulant activity. Furthermore, cytoskeletons bind Factor Xa with a dissociation constant of 10.4×10^{-10} M, a value more consistent for platelet Factor Va than for Factor V. Finally, it is postulated that Factor V arises on the platelet cytoskeleton from a site on the surface of the platelet. This platelet surface is further postulated to bind

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Tuszynski, G. P., W

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Tuszynski, G. P., Be

Blood 59(6):1148-115

Other support: U. S.

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Factor Va, Factor Xa, and Ca^{2+} and form the crucial prothrombinase complex that generates thrombin, the penultimate step in fibrin formation.

Tuszynski, G. P., *Walsh, P. N.*, Piperno, J. R., and Koshy, A.

The Journal of Biological Chemistry 257(8): 4557-4563, 1982.

Other support: Department of Health and Human Services.

From the Specialized Center on Thrombosis Research, Temple University School of Medicine, Philadelphia.

FACTOR XI ANTIGEN AND ACTIVITY IN HUMAN PLATELETS

The existence and physiologic relevance of platelet factor XI has been a matter of controversy for about 12 years now. In the work presented here, washed platelets, contaminated with less than 0.20% plasma factor XI, were examined for the presence of factor XI antigen and activity. These platelets contained a factor-XI-like coagulant activity (0.67 ± 0.11 U/10^{11} platelets) that remained constant after successive washings. By means of indirect immunofluorescence, a monospecific antibody to factor XI showed specific staining of both normal platelets and platelets from patients deficient in plasma factor XI. Radiolabeled Triton extracts of washed platelets and labeled purified factor XI solutions were analyzed for factor XI antigen by Staph A immunoprecipitation analysis using antibody to purified factor XI followed by SDS gel electrophoresis. On unreduced gels, the platelet material ran as a single band having an apparent molecular weight of 220,000 daltons, whereas purified plasma factor XI gave a single band at 160,000 daltons. On reduced gels, the platelet material analyzed as a single band at 52,000 daltons, whereas purified factor XI gave a single band of 80,000 daltons. Analysis of a partially purified factor XI preparation from platelets by immunoelectrophoresis revealed that the platelet preparation displayed a slightly lower cathodal electrophoretic mobility at pH 8.6 than did plasma factor XI and yet appeared to possess complete antigenic identity with plasma factor XI. These results indicate that platelets possess a form of factor XI that exists as a disulfide-linked 52,000-dalton tetramer in contrast to the plasma form that circulates as a 80,000-dalton disulfide-linked dimer.

Tuszynski, G. P., Bevacqua, S. J., Schmaier, A. H., Colman, R. W., and *Walsh, P. N.*

Blood 59(6):1148-1156, 1982.

Other support: U. S. Department of Health and Human Services.

From the Specialized Center on Thrombosis Research, Temple University School of Medicine, Philadelphia.

INCREASED CHOLESTEROL BIOSYNTHESIS IN LEUKEMIC CELLS FROM PATIENTS WITH HAIRY CELL LEUKEMIA

Leukemic cells from patients with hairy cell leukemia (HCL), a chronic B-cell leukemia, have an increased cholesterol content when compared with normal human

peripheral blood mononuclear cells (PBMNC). In the studies presented here it is shown that HCL cells synthesize ^{14}C-cholesterol from ^{14}C-acetate precursor in lipoprotein-depleted medium at a rate that is 5-6-fold higher than PBMNC. The increased rate of cholesterol synthesis in HCL cells by comparison with PBMNC persists despite the presence of 100 μg low density lipoprotein (LDL), although cholesterol synthesis in HCL cells and PBMNC is suppressed 60%-70% under these conditions, suggesting that HCL cells possess a normal LDL receptor mechanism. HCL cells mount a subnormal DNA synthetic response to Con A when compared with PBMNC and also show a significantly lower increase in cholesterol biosynthesis in response to Con A exposure. Increased cholesterol synthesis in HCL cells is found in both splenectomized HCL patients and those with intact spleens. It was also shown here that increased cholesterol synthesis in HCL cells cannot be explained by increased loss of newly synthesized cholesterol into the culture medium. In fact, HCL cells retain such cholesterol more avidly than do PBMNC. In a related experiment it was seen that HCL cells and PBMNC are both equally sensitive to cholesterol synthesis inhibition by ML-236B and 25-hydroxycholesterol, but are approximately 10 times more sensitive to ML-236B than are bone marrow MNC. The increased rate of cholesterol synthesis in HCL cells may contribute to their redundant plasma membrane. It seems, therefore, that pharmacologic suppression of cholesterol synthesis in HCL cells could be useful in reversing the abnormal surface properties of these cells.

Yachnin, S. et al.

Blood 61(1):50-60, 1983.

Other support: Nalco Cancer Research Fund, the Leukemia Research Foundation, National Institutes of Health, National Cancer Institute, and the Levin Family Research Fund.

From the Department of Medicine and the Committee on Immunology, University of Chicago School of Medicine, Chicago.

MEVALONIC ACID IN CONJUNCTION WITH HELP FROM NEUTROPHILS INDUCES DNA SYNTHESIS AND CELL CYCLING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Mevalonic acid plays an important role in the regulation of mammalian cell growth and division. Results from earlier studies have even suggested a critical role for mevalonic acid, independent of its conversion to cholesterol, in the regulation of DNA synthesis and cell replication. Evidence presented in this paper shows that mevalonic acid does indeed stimulate DNA synthesis in human peripheral blood lymphocytes which have been isolated by gravity sedimentation of blood and freed of adherent cells by nylon column passage. Human peripheral blood mononuclear cells isolated by the Ficoll-Hypaque technique respond less well, but their response to mevalonic acid can be enhanced by the neutrophil-rich Ficoll-Hypaque-isolated "bottom" cell fraction. The kinetics of mevalonic acid-induced lymphocyte transformation are simlar to those of more classic lymphocyte mitogens. In addition to stimulating lymphocyte DNA synthesis, mevalonic acid produces a population of cells representing all phases of the cell cycle whose morphological characteristics are typical of those seen with more conventional mitogens. The DNA synthetic response of lymphocytes to mevalonic

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acid can be abolished by prior exposure of the lymphocytes to X-irradiation or mitomycin C, while the helper effect of granulocytes is unaffected by either treatment. These observations suggest that mevalonic acid may play a role as a critical substance which supports the propagation of cells programmed to divide, or stimulated to divide by various initiators of cell growth. Also, in susceptible cell populations, mevalonic acid may act as an inducer of the entire program of the cell cycle.

Yachnin, S. and Richman, D. P.

Cellular Immunology 72:248-262, 1982

Other support: U.S. Public Health Service, Muscular Dystrophy Association, and the Nalco Research Foundation.

From the Departments of Medicine and Neurology, Franklin McLean Memorial Research Institute, and the Committee on Immunology, University of Chicago School of Medicine, Chicago.

INHIBITION OF OXYGENATED STEROL ENTRY INTO HUMAN RED CELLS AND LYMPHOCYTES BY ISOLATED SERUM LIPOPROTEINS

The effects of human lipoproteins on the entry of oxygenated sterols into red-cell membranes were studied here using purified lipoprotein fractions. Also, in an attempt to compare this process with that in red cells, the characteristics of oxygenated sterol entry into nucleated human cells and its modulation by serum lipoproteins were studied. Results of these studies show that the uptake of oxygenated sterols by nucleated human cells is comparable in almost every respect to the uptake of oxygenated sterols by human red-cell membranes. The uptake in the two tissues is modulated to a similar extent by such variables as concentration of oxygenated sterol in the incubation medium, temperature, time of exposure, the specific sterol studied, and the lipoprotein composition of the medium in which cellular exposure to oxygenated sterol occurs. In addition, the rank order of efficiency with which various oxygenated sterols enter red cells and lymphocytes is the same, with 7β-hydroxycholesterol entering most efficiently and 25-hydroxycholesterol least efficiently. Both low density lipoproteins (LDL) and high density lipoproteins (HDL), when added to oxygenated sterol-containing medium, effectively diminish the amount of oxygenated sterol taken up by red cells and lymphocytes; LDL is approximately 2.5-4 times more efective than HDL in preventing oxygenated sterol entry into cells. Since oxygenated sterols have been reported to be atherogenic, the modulating effects of lipoproteins on oxygenated sterol uptake by cells, and the alterations in membrane structure and function which ensue, may be a useful model for further study.

Yachin, S., Chung, J. and Scanu, A. M.

Biochimica et Biophysica Acta 713:538-546, 1982.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Departments of Medicine and Biochemistry, and the Committee on Immunology, the University of Chicago, Chicago.

MEVALONIC ACID AS AN INITIATOR OF CELL GROWTH: STUDIES USING HUMAN LYMPHOCYTES AND INHIBITORS OF ENDOGENOUS MEVALONATE BIOSYNTHESIS

Mevalonic acid, which is unique in being the only lymphocyte mitogen known which is also a low molecular weight organic product of normal mammalian cell intermediary metabolism, plays a role in the regulation of mammalian cell replication. In the work reported here, it is shown specifically that mevalonic acid (5×10^{-4}-1×10^{-2} M) stimulates DNA synthesis, morphologic transformation and cell cycling in peripheral blood human lymphocytes. Other organic acid anions which serve as cholesterol and mevalonate precursors are devoid of such effects. Both ML-236B and 25-hydroxycholesterol, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, inhibit concanavalin A-induced lymphocyte transformation, but only the inhibition of ML-236B can be overcome by exogenous mevalonate. In contrast, only 25-hydroxycholesterol inhibits mevalonate-induced lymphocyte DNA synthesis. The effects of mevalonic acid on lymphocytes cannot be reproduced by isopentenyl adenine or isopentenyl adenosine. Unregulated endogenous cellular synthesis of mevalonic acid may contribute to uncontrolled growth in certain malignant cell lines.

Yachin, S.

Oncodevelopmental Biology and Medicine 3:111-123, 1982.

Other support: The Leukemia Research Foundation, U. S. Department of Energy, U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine, Franklin McLean Memorial Research Institute, and the Committee on Immunology, the University of Chicago, Chicago.

CHOLESTEROL AND MEVALONIC ACID ARE INDEPENDENT REQUIREMENTS FOR THE *IN VITRO* PROLIFERATION OF HUMAN BONE MARROW GRANULOCYTE PROGENITOR CELLS: STUDIES USING ML-236B

ML-236B (compactin) is a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key regulatory enzyme in the sequence that catalyzes the conversion of acetate to mevalonic acid in cholesterol biosynthesis. This compound causes marked inhibition of human bone marrow granulocyte progenitor cell (CFU-C) proliferation. In the work presented here, the effects of ML-236B on the proliferation of CFU-C *in vitro* were investigated and compared with the effects of 25-hydroxycholesterol, the most potent oxygenated sterol compound studied previously. Also, the action of ML-236B was compared with that of 25-hydroxycholesterol on sterol synthesis in marrow mononuclear cells and the reversibility of the effects of ML-236B by mevalonic acid and cholesterol was examined in an attempt to determine the precise mechanism of inhibition of CFU-C growth by ML-236B. Overall, results from these studies showed that: (1) ML-236B is a potent inhibitor of CFU-C proliferation, DNA synthesis, and cholesterol biosynthesis from acetate precursor in marrow mononuclear cells; (2) the effects of ML-236B are completely reversed by mevalonic acid but not by cholesterol, suggesting that mevalonic acid *per se* or one or more of its nonsterol products is critical for cell growth; (3) the inhibitory effects of 25-hydroxycholesterol on CFU-C proliferation and cholesterol biosynthesis are not solely a result

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Hoffman, P. C., Ri

Blood 61(4):667-67

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Hoffman, P. C., Richman, C. M., Larson, R. A., and *Yachnin, S.*

Blood 61(4):667-671, 1983.

Other support: Leukemia Research Foundation, the Nalco Cancer Center Research Fund, U. S. Public Health Service, and the National Institutes of Health.

From the Department of Medicine and the Committee on Immunology, University of Chicago, Chicago.

CYTOCHALASIN B IS A POTENT MITOGEN FOR CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IN VITRO

Cytochalasin B, a fungal metabolite, has been shown to be a potent mitogen for malignant lymphocytes from patients with chronic lymphocytic leukemia (CLL). When peripheral blood lymphocytes from 19 patients with CLL of B cell origin (B-CLL) were cultured with 0.5 μg cytochalasin B/ml, significant new DNA synthesis ([^{14}C]thymidine incorporation) occurred in 18. Stimulation indices with cytochalasin B varied widely, but in 11 cases they exceeded those seen with concanavalin A (Con A), phytohemagglutinin, or pokeweed mitogen. In all 11, the mitogenic response to cytochalasin B exceeded that to pokeweed mitogen, which is believed to be a T cell-dependent B cell mitogen. In three cases, the responses to cytochalasin B were 8.6, 3.5, and 2.3 times greater than those to Con A. As with other mitogens, the DNA synthetic response to cytochalasin B was time and dose dependent. Significant mitogenic effects were observed with 0.1-5 μg cytochalasin B/ml with a peak of 0.5-2 μg/ml. Stimulated DNA synthesis was abolished by 1 mM hydroxyurea. Cells from two patients with B-CLL were separated by rosetting with sheep erythrocytes (E). Depletion of E-rosette-positive cells from the CLL cell population abolished the response to Con A but did not affect the response to cytochalasin B. As could be seen here, cytochalasin B is a potent mitogen for B-CLL cells and may be useful in cytogenetic studies of this often indolent neoplasm.

Larson, R. A. and *Yachnin, S.*

Journal of Clinical Investigation 72:1268-1276, 1983.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, and the Franklin McLean Memorial Research Institute, Chicago.

RATES OF CHOLESTEROL BIOSYNTHESIS ARE RELATED TO EARLY DIFFEREINTIATION IN ACUTE NONLYMPHOCYTIC LEUKEMIA CELLS

The capacity of leukemic cells from 20 patients with acute nonlymphocytic leukemia to synthesize cholesterol from acetate was examined in this study. When the leukemic cell population was classified into subsets (French-American-British [FAB] classification) based on morphology, cytochemistry and cytogenetics, marked differences in the cholesterol biosynthesis rates of each subset were found. As leukemic

cells differentiated along myeloid (acute promyelocytic) or monocytoid (acute myelomonocytic) pathways, their cholesterol-synthesis rates diverged and approached those of their respective mature cellular counterparts, the neutrophil or the peripheral blood monocyte. Enhanced sterol synthesis in leukemic cells could not be explained by more rapid efflux of membrane cholesterol to the environment. In addition, the different rates of cholesterol biosynthesis in leukemic-cell subgroups did not correlate with differences in their rates of cellular DNA synthesis. As could be seen from the material presented in this paper, the normal divergence of sterol-synthesizing capacity found between mature neutrophils and monocytes develops at an early stage of differentiation and is detectable even in leukemic cells.

Yachnin, S., Larson, R. A. and West, E. J.

British Journal of Haematology 54:459-466, 1983.

Other support: U. S. Public Health Service and the Nalco Cancer Research Center.

From the Department of Medicine and Committee on Immunology, the University of Chicago, Chicago.

IV. *Neuropharmacology and Physiology*

GENETICS OF NICOTINE RESPONSE IN FOUR INBRED STRAINS OF MICE

Nicotine, which is known to have multiple effects, appears to act at the neuromuscular junction, at the autonomic ganglia and in the brain. The actions of nicotine also appear to be influenced by genetic factors. In the present attempt to assess these genetic influences, the effects of nicotine on five behavioral and physiological measures were determined in four inbred mouse strains (BALB, C57BL, DBA and C3H). In addition, the binding characteristics of nicotine and α-bungarotoxin (α-BTX), two ligands which appear to label different nicotinic receptors, were measured in seven discrete brain regions as well as in whole brain. Results showed that a number of differences in response to nicotine were found among the four inbred strains. Whereas nicotine depressed open-field activity of BALB, C57BL and DBA mice in a dose-dependent manner, low doses of nicotine increased locomotor activity in C3H mice. The doses of nicotine tested reduced Rotarod performance in DBA and C57BL mice but not in C3H and BALB mice. All four strains displayed a dose-dependent decrease in body temperature after nicotine administration. The BALB mice were more sensitive to the agent than were the C3H, while the effects on C57BL and DBA mice were intermediate. All four strains showed a transient increase in respiration only after a high (2.0 mg/kg) nicotine dose. No dose of nicotine was found to have an effect on the startle response after auditory stimulation in three of the strains; only the C3H mice exhibited enhanced startle after nicotine administration. Measurement of the affinity and density of nicotine and α-BTX binding sites provided no clear explanation for the differences among the strains. The affinity of nicotine for either the DL-[^{3}H] or α-BTX binding sites did not vary from strain to strain or from region to region; however, several differences in the density of α-BTX binding sites were detected in the hippocampus and midbrain. Thus, genetic factors influence response to nicotine, but variation in response is not easily explained by differences in brain nicotinic receptors.

Marks, M. J., Burch, J. B. and *Collins,*

The Journal of Pharmacology and Expe

Other support: National Institutes of He

From the Institute for Behavioral Genet Colorado, Boulder.

QUANTITATION OF TOLERANCE D OXOTREMORINE TREATMENT

An earlier study concerning the effec clear evidence of the development of tole used to evaluate tolerance in that stud tolerance development. For the study pre to quantitate the tolerance which develo muscarinic agonist. Cumulative dose-res of oxotremorine on body temperature ar quential injections to individual animals. ably to those constructed by injecting indi sequential injection technique was used t ment to oxotremorine. A linear relationsh and magnitude of change of the ED_{50} valu observed, with animals receiving an infu increase in ED_{50}. Dose-response curves constructed for naive animals. The oxc temperature to 35°C was 80-fold greater t kg/hr. Time courses of recovery from a c change in metabolism occurring during c sion resulted in a decrease in the total nur low-affinity sites.

Marks, M. J., Artman, L. D. and *Collin*

Pharmacology Biochemistry & Behavior

Other support: Research Scientist Devel

From the Institute for Behavioral Genetics Center, University of Colorado, Boulder

INTRAVENOUS INJECTIONS OF NIC DISCRETE REDUCTIONS OF HYPOT LEVELS ASSOCIATED WITH INCRE PROLACTIN SECRETION

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Marks, M. J., Burch, J. B. and *Collins, A. C.*

The Journal of Pharmacology and Experimental Therapeutics 226(1):291-302, 1983.

Other support: National Institutes of Health.

From the Institute for Behavioral Genetics and School of Pharmacy, University of Colorado, Boulder.

QUANTITATION OF TOLERANCE DEVELOPMENT AFTER CHRONIC OXOTREMORINE TREATMENT

An earlier study concerning the effects of chronic oxotremorine infusion provided clear evidence of the development of tolerance to oxotremorine. However, the method used to evaluate tolerance in that study did not permit a quantitative analysis of tolerance development. For the study presented here, a new procedure was developed to quantitate the tolerance which develops as mice are chronically infused with the muscarinic agonist. Cumulative dose-response curves were constructed for the effects of oxotremorine on body temperature and rotarod performance by administering sequential injections to individual animals. These dose-response curves compare favorably to those constructed by injecting individual animals with one of several doses. The sequential injection technique was used to assess the magnitude of tolerance development to oxotremorine. A linear relationship between oxotremorine infusion rate (dose) and magnitude of change of the ED_{50} value for impairment of rotarod performance was observed, with animals receiving an infusion rate of 1.0 mg/kg/hr showing a 24-fold increase in ED_{50}. Dose-response curves for tolerant animals were parallel to those constructed for naive animals. The oxotremorine dose required to decrease body temperature to 35°C was 80-fold greater than control in the group treated with 1.0 mg/kg/hr. Time courses of recovery from a challenge dose of oxotremorine suggest little change in metabolism occurring during chronic infusion. Chronic oxotremorine infusion resulted in a decrease in the total number of QNB binding sites in both high- and low-affinity sites.

Marks, M. J., Artman, L. D. and *Collins, A. C.*

Pharmacology Biochemistry & Behavior 19:103-113, 1983.

Other support: Research Scientist Development Award.

From the Institute for Behavioral Genetics, School of Pharmacy and Alcohol Research Center, University of Colorado, Boulder.

INTRAVENOUS INJECTIONS OF NICOTINE INDUCE VERY RAPID AND DISCRETE REDUCTIONS OF HYPOTHALAMIC CATECHOLAMINE LEVELS ASSOCIATED WITH INCREASES OF ACTH, VASOPRESSIN AND PROLACTIN SECRETION

Previous studies have indicated that the various types of hypothalamic catecholamine (CA) nerve terminal systems are regulated by nicotine-like cholinergic receptors. In view of the rapid onset of action of nicotine on the central nervous system, it seemed of interest at this time to analyze the action of nicotine on the hypothalamic CA and on neuroendocrine parameters such as prolactin, ACTH, vasopressin and corticos-

terone serum levels 2, 4 and 30 min. after an intravenous injection. Results presented here show that nicotine given i.v. can, within minutes, induce increases of ACTH, vasopressin and prolactin secretion in the male rat, giving further evidence for the existence of nicotine-like cholinergic receptors involved in the regulation of these hormones. These changes were associated with rapid reductions of noradrenaline (NA) levels in the subependymal layer of the median eminence, in the nuc. dorsomedialis hypothalami and in the anterior and posterior periventricular hypothalamic area as revealed by quantitative microfluorimetrical measurements of CA fluorescence. Intraindividual correlations indicate the involvement of an inhibitory noradrenergic mechanism in the subependymal layer of the median eminence in the regulation of ACTH secretion, the involvement of noradrenergic mechanisms in the posterior periventricular area in the regulation of prolactin secretion and the involvement of dopaminergic mechanisms in the medial palisade zone of the median eminence in the regulation of prolactin secretion. A rapid rise of prolactin secretion seems to be associated mainly with a reduction of NA levels in the posterior periventricular area indicating the existence of possible facilitatory noradrenergic mechanism in this region regulating prolactin secretion.

Andersson, K., Siegel, R., *Fuxe, K.*, and Eneroth, P.

Acta Physiologica Scandinavica 118(1):35-40, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet, Stockholm; Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden.

INVOLVEMENT OF CHOLINERGIC NICOTINE-LIKE RECEPTORS AS MODULATORS OF AMINE TURNOVER IN VARIOUS TYPES OF HYPOTHALAMIC DOPAMINE AND NORADRENALINE NERVE TERMINAL SYSTEMS AND OF PROLACTIN, LH, FSH AND TSH SECRETION IN THE CASTRATED MALE RAT

This paper provides evidence that repeated high subcutaneous doses of nicotine in the castrated male rat can reduce the amine levels in various hypothalamic dopamine (DA) and noradrenaline (NA) nerve terminal systems. Specifically, the effects of high repeated subcutaneous doses (4 × 2 mg/kg) of nicotine were evaluated on DA and NA levels and turnover in the long-term castrated male rat using catecholamine (CA) fluorescence histochemistry in combination with quantitative microfluorometry. The CA turnover was evaluated by studying the decline of the CA stores following tyrosine hydroxylase inhibition using α-methyltyrosine methyl ester (H 44/68). In the same experiments trunk blood was collected for the determination of serum prolactin, LH, FSH and TSH levels using standard radioimmunoassay procedures. The nicotine treatment produced a significant depletion of CA stores and an increase of CA turnover in DA and NA nerve terminals of the median eminence and in peri- and paraventricular NA systems. These effects were significantly counteracted by pretreatment with mecamylamine. Nicotine significantly reduced serum prolactin and TSH levels, and after H 44/68 it also reduced LH and FSH serum levels. Many of these actions were counteracted by mecamylamine pretreatment. Overall intraindividual correlations showed a significant correlation between reduced CA turnover in several hypothalamic areas and

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hypothalamic nucleus and increased
median eminence and increased ser

Andersson, K., *Fuxe, K.*, Eneroth,

Acta Physiologica Scandinavica 11(

Other support: Svenska Tobaks AI

From the Department of Histology,
partment of Obstetrics and Gynecc
and the Department of Human Phy

INTERACTIONS OF NICOTINE
REGULATION OF TELENCEPH
CATECHOLAMINE LEVELS AN
ADENOHYPOPHYSEAL HORM
RAT

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Andersson, K., *Fuxe, K.*, Eneroth

Naunyn-Schmiedeberg's Archives

Other support: Svenska Tobaks A

From the Department of Histology
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pital, Stockholm, Sweden, and th
Modena, Modena, Italy.

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Stockholm; Laboratories of
, Department of Neurology,
Hormone Laboratory, De-
pital, Stockholm, Sweden.

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increased serum LH and FSH levels, increased NA turnover in the paraventricular hypothalamic nucleus and increased serum TSH levels, and reduce DA turnover in the median eminence and increased serum LH levels.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Acta Physiologica Scandinavica 116:41-50, 1982.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet; Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden, and the Department of Human Physiology, University of Modena, Modena, Italy.

INTERACTIONS OF NICOTINE AND PENTOBARBITONE IN THE REGULATION OF TELENCEPHALIC AND HYPOTHALAMIC CATECHOLAMINE LEVELS AND TURNOVER AND OF ADENOHYPOPHYSEAL HORMONE SECRETION IN THE NORMAL MALE RAT

The way(s) that nicotine may interact with pentobarbitone, a sedative hypnotic drug, in the regulation of activity in the different central dopamine (DA) and noradrenic (NA) pathways was evaluated in this study. To do this, the effects of single intraperitoneal injections of nicotine (1 mg/kg) and pentobarbitone (30 mg/kg) alone or in combination were studied on catecholamine (CA) nerve terminal system of the hypothalamus and the forebrain and on the adenohypophyseal hormone secretion of the normal male rat. Results showed that nicotine produced discrete reductions of DA levels and increases of DA turnover in striatal and limbic areas of the forebrain and increases of amine turnover in different hypothalamic NA nerve terminal systems. These effects were all antagonized by simultaneous treatment with pentobarbitone. Pentobarbitone alone, however, did not modulate CA levels or turnover in the various parts of the hypothalamus and forebrain analyzed. On the other hand, pentobarbitone increased GH and prolactin secretion and in association with tyrosine hydroxylase inhibition markedly reduced corticosterone secretion. These effects were partly counteracted by nicotine in the case of GH and prolactin secretion. Furthermore, a positive interaction appears to exist between nicotine and pentobarbitone in their actions on LH secretion. These results suggest, therefore, that pentobarbitone can antagonize the actions of nicotine on CA levels and turnover in various CA nerve terminal systems of the brain leading to possible reductions in nicotine-induced arousal and positive reinforcement. The neuroendocrine actions of pentobarbitone do not seem to be greatly modulated through nicotinic cholinergic receptors.

Andersson, K., *Fuxe, K.*, Eneroth, P., Agnati, L. F., and Gustafsson, J.-A.

Naunyn-Schmiedeberg's Archives of Pharmacology, 321:287-291, 1982.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology and Medical Nutrition, Karolinska Institutet, and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden, and the Department of Human Physiology, University of Modena, Modena, Italy.

IMMOBILIZATION STRESS-INDUCED CHANGES IN DISCRETE HYPOTHALAMIC CATECHOLAMINE LEVELS AND TURNOVER, THEIR MODULATION BY NICOTINE AND RELATIONSHIP TO NEUROENDOCRINE FUNCTION

Male Sprague-Dawley rats were used in this evaluation of immobilization stress–nicotine interactions on hypothalamic catecholamine (CA) systems, and on neuroendocrine function, as manifested by the secretion of vasopressin, ACTH, corticosterone (CS), prolactin (PRL), LH and FSH. Immobilization stress (1 h) induced discrete reductions in noradrenaline (NA) levels in the posterior periventricular hypothalamic region and in the paraventricular hypothalamic nucleus, and a decrease in dopamine (DA) turnover in the medial palisade zone (MPZ) of the median eminence, but failed to induce regional increases of hypothalamic NA turnover. Stress also stimulated the secretion of ACTH, CS and PRL, while vasopressin, LH and FSH serum levels were unaffected. The stress-induced reduction of DA turnover in MPZ may mediate the stress-induced increase of PRL secretion. Nicotine did not by itself significantly influence CA turnover in the various CA nerve terminal systems analyzed in the hypothalamus, but it reduced NA levels in the subependymal layer of the median eminence. These changes in NA levels in discrete hypothalamic regions may be correlated with the stress-induced secretion of ACTH, since nicotine treatment to some extent counteracted the stress-induced changes in CA activity and in ACTH, but not in PRL secretion at the 1 h time interval studied. However, a stress-induced reduction of DA turnover in MPZ may be involved in causing the stress-induced increase of PRL secretion.

Fuxe, K. et al.

Acta Physiologica Scandinavica 117:421-426, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Department of Histology, Karolinska Institutet, Stockholm; the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden; Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; and the Department of Human Physiology and Endocrinology, University of Modena, Modena, Italy.

RAPID AND DISCRETE CHANGES IN HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS INDUCED BY AUDIOGENIC STRESS, AND THEIR MODULATION BY NICOTINE–RELATIONSHIP TO NEUROENDOCRINE FUNCTION

The time course of audiogenic stress-induced changes in discrete hypothalamic dopamine (DA) and noradrenaline (NA) nerve terminal systems was studied using quantitative microfluorometric evaluations of catecholamine stores and radioimmunoassays for the determination of serum hormone levels, and was correlated with simultaneous stress effects upon the secretion of ACTH, corticosterone (CS), prolactin (PRL), and vasopressin. Finally, the effects of nicotine upon these parameters were evaluated alone or in association with stress. Audiogenic stress and nicotine induced very rapid and discrete decreases in NA levels in the subependymal layer (SEL), in the

parvocellular part of nuc. paraventricular periventricular hypothalamic systems, (P utes following the onset of treatment. Ir served after nicotine treatment but could r changes in catecholamine levels. These nicotine- and stress-induced increases c secretion found in these experiments. Stre vasopressin secretion induced by nicoti which stress combined with smoking can arterial blood pressure and finally to sust

Siegel, R. A., Andersson, K., *Fuxe, K.*, F

European Journal of Pharmacology 91(1

Other support: Svenska Tobaks AB, St

From the Laboratories of Experimental E Department of Neurology, Hadassah Un ment of Histology, Karolinska Institutet, Obstetrics and Gynecology, Karolinska of Human Physiology and Endocrinolog Research Laboratories, Astra Läkemede

EFFECTS OF PRENATAL ADMINIST ACID POOLS, PROTEIN METABOLI BRAIN

The material presented here indicate function, and shows that the processes newborn rat brain are affected by nicot effects of nicotine on brain protein met binding site in newborn animals expose synthesis rates measured *in vivo* were l Protein degradation rates measured *in vi* 13%. The effect was specific for L-(−)ni nicotinamide had no effect on degradat mainly nonessential amino acids and ar tion, were changed somewhat; an incr (21%), serine (35%) and glycine (35%) in the offspring of nicotine treated anim decrease in protein metabolism. Nicotin of animals exposed to nicotine during treatment of adult rats with nicotine, i binding site changed with age.

Sershen, H., Reith, M. E. A., Banay-S

Neurochemical Research 7(12):1515-152

From the Center for Neurochemistry, New York.

parvocellular part of nuc. paraventricularis hypothalamic (PA FP) and in the posterior periventricular hypothalamic systems, (PV II); the decreases were apparent two minutes following the onset of treatment. Increases of arterial blood pressure were observed after nicotine treatment but could not have been a major factor in producing the changes in catecholamine levels. These changes in NA levels may be related to the nicotine- and stress-induced increases of ACTH (SEL and PA FP) and prolactin secretion found in these experiments. Stress enhanced the rapid but variable increase in vasopressin secretion induced by nicotine, suggesting one possible mechanism by which stress combined with smoking can contribute to the development of increased arterial blood pressure and finally to sustained hypertension.

Siegel, R. A., Andersson, K., *Fuxe, K.*, Eneroth, P., Lindbom, L-O, and Agnati, L. F.

European Journal of Pharmacology 91(1):49-56, 1983.

Other support: Svenska Tobaks AB, Stockholm, Sweden.

From the Laboratories of Experimental Endocrinology and Experimental Neurology, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; Department of Histology, Karolinska Institutet, and the Hormone Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm, Sweden; Departments of Human Physiology and Endocrinology, University of Modena, Modena, Italy; and Research Laboratories, Astra Läkemedel AB, Södertälje, Sweden.

EFFECTS OF PRENATAL ADMINISTRATION OF NICOTINE ON AMINO ACID POOLS, PROTEIN METABOLISM, AND NICOTINE BINDING IN THE BRAIN

The material presented here indicates that nicotine has diverse influences on brain function, and shows that the processes of protein degradation and synthesis in fetal newborn rat brain are affected by nicotine. Specifically, this study investigated the effects of nicotine on brain protein metabolism and on the properties of the nicotine binding site in newborn animals exposed to nicotine during gestation. Brain protein synthesis rates measured *in vivo* were lower by 18% in newborn of treated animals. Protein degradation rates measured *in vitro* in the presence of nicotine were lower by 13%. The effect was specific for L-(−)nicotine since D-(+)nicotine, nicotinic acid, or nicotinamide had no effect on degradation rates. Newborn brain amino acid levels, mainly nonessential amino acids and amino acids of putative neurotransmitter function, were changed somewhat; an increase in the level of taurine (13%), threonine (21%), serine (35%) and glycine (35%) and a decrease in lysine (14%) were observed in the offspring of nicotine treated animals. These changes could not account for the decrease in protein metabolism. Nicotine binding was higher by 25% in the offspring of animals exposed to nicotine during gestation. No such increase was found after treatment of adult rats with nicotine, indicating that the properties of the nicotine binding site changed with age.

Sershen, H., Reith, M. E. A., Banay-Schwartz, M., and *Lajtha, A.*

Neurochemical Research 7(12):1515-1522, 1982.

From the Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York.

V. *Pharmacology and Biochemistry*

DIFFERENTIAL SCANNING CALORIMETRY OF α_2-MACROGLOBULIN AND α_2-MACROGLOBULIN-PROTEINASE COMPLEXES

α_2-macroglobulin (α_2M), a major component of serum, appears to play a role in regulating the activity and concentration of endoproteinases since it has the capability of sequestering endoproteinases of all four major classes. In the study presented here, differential scanning calorimetry was shown to detect substantial structural alterations occurring on the association of proteinases with the serum glycoprotein α_2M. At pH 7.5, the thermally induced unfolding of the macroglobulin occurs at approximately 60° C with a transition enthalpy of 17J/g. Association of active thermolysin, trypsin and papain shifts the transition temperature to 77°C (transition enthalpy 5J/g), indicating that a substantial conformational change accompanies the binding event. The stoicheiometry of the thermolysin-α_2-macroglobulin association producing this change appears to be unity, implying the presence of cooperative subunit interactions in the mechanism of association. The calorimetric method provides a novel approach for the evaluation of conformational variants induced on protein-protein association or preexisting in the purified macroglobulin. As could be seen here, differential scanning calorimetry permits the ready detection of changes in the overall protein structure and is thus complementary to chemical and spectroscopic methods of analysis. In principle, the method can be employed to monitor behavior of α_2M in solutions more closely approximating the situation *in vivo* (*e.g.* plasma).

Chlebowski, J. F. and Williams, K.

Biochemical Journal 209:725-730, 1983.

Other support: National Institutes of Health.

From the Department of Biochemistry, Virginia Commonwealth University, Medical College of Virginia, Richmond.

THE SENSITIZATION OF THE TOXICITY OF SUPEROXIDE AND VITAMIN C BY COPPER AND IRON: A SITE SPECIFIC MECHANISM

In the study presented here, radiolysis of air-saturated solutions of sodium formate was used to generate superoxide. (This method yields superoxide as the predominant radical and seems to be the "cleanest" way to generate superoxide.) When superoxide's effect on a purified enzyme (penicillinase) and on bacteriophages T4 and T7 was studied in the absence of copper, it was found that the superoxide radicals were practically inert. However, when traces of Cu(II) were added, a dramatic enhancement of the damage with a dose modifying factor was observed. A similar copper-induced effect was noted when vitamin C instead of superoxide was added to air-saturated solutions of the enzyme acetylcholine esterase, bacteriophage T7 or the bacterium *Escherichia coli*. Experiments with isotopically labeled phages indicated that treating the phages with ascorbate and copper impaired their adsorption to the host, the injection of their DNA, as well as the expression of their genetic information within the host. Many of the observations discussed in this paper seem to be in agreement with a site specific or site directed mechanism. This mechanism, which may operate in many, though not necessarily all, biologic bound at or near sensitive sites.

Czapski, G. et al.

In: Cohen, G. and Greenwald, R. *tems*, New York: Elsevier Scienc *Aspects*, pp. 111-115.

Other support: Department of E Neuherberg, Federal Republic of C

From the Departments of Physical chemistry, the Hebrew University.

INACTIVATION OF T-EVEN AI SUPEROXIDE OR VITAMIN C

In this comparative study, the damage caused to various phages l difference was found in the respons bacteriophages were highly sensitiz the T-even phages appeared far mo addition of 10^{-7}M copper resulted i fying Factor >80. Lambda phag similar response. Polyethyleneglyc age mediated by superoxide and co the phages were irradiated in the ab in the sensitivities of the T-even a radiolytically formed O_2^- radicals c treatment with copper and vitami appears likely that the mechanism of copper to target biomolecules, radicals or vitamin C.

Aronovitch, J., Samuni, A. and C

In: Cohen, G. and Greenwald, R. *tems*, New York: Elsevier Scien *Aspects*, pp. 380-382.

Other support: Department of E Neuherberg, Federal Republic of

From the Departments of Molecu University, Jerusalem, Israel.

-BUNGAROTOXIN LABELIN LOCALIZATION AT THE MAU HATCHETFISH

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though not necessarily all, biological systems, assumes that copper and iron ions are bound at or near sensitive sites.

Czapski, G. et al.

In: Cohen, G. and Greenwald, R. A. (eds.): *Oxy Radicals and their Scavenger Systems*, New York: Elsevier Science Publishing Co., Inc., 1983, vol. I: *Molecular Aspects*, pp. 111-115.

Other support: Department of Energy and Gesellschaft für strahlen Forschung, Neuherberg, Federal Republic of Germany.

From the Departments of Physical Chemistry, Molecular Biology and Cellular Biochemistry, the Hebrew University, Jerusalem, Israel.

INACTIVATION OF T-EVEN AND T-ODD COLIPHAGES INDUCED BY SUPEROXIDE OR VITAMIN C AND SENSITIZED BY COPPER

In this comparative study, the sensitizing effect of copper (II) ions on the radiation damage caused to various phages by superoxide radicals was noted, and a surprising difference was found in the response of T4 and T2 as compared to that of T7 phages: T7 bacteriophages were highly sensitized by copper towards superoxide radicals, whereas the T-even phages appeared far more resistant to this copper effect. With T7 phages the addition of 10^{-7}M copper resulted in a strikingly large sensitization with a Dose Modifying Factor >80. Lambda phages, which resemble the T-odd series, exhibited a similar response. Polyethyleneglycol did not provide any protection against the damage mediated by superoxide and copper. This is in contrast to the effect observed when the phages were irradiated in the absence of copper. In addition, the marked difference in the sensitivities of the T-even and T-odd phages to the copper-mediated effect of radiolytically formed O_2^- radicals closely paralleled the response of these phages to treatment with copper and vitamin C. Overall, based on these and other studies, it appears likely that the mechanism of sensitization by copper ions involves the binding of copper to target biomolecules, followed by their reduction by either superoxide radicals or vitamin C.

Aronovitch, J., Samuni, A. and *Czapski, G.*

In: Cohen, G. and Greenwald, R. A. (eds.): *Oxy Radicals and their Scavenger Systems*, New York: Elsevier Science Publishing Co., Inc., 1983, vol. I: *Molecular Aspects*, pp. 380-382.

Other support: Department of Energy and Gesellschaft für Strahlen Forschung, Neuherberg, Federal Republic of Germany.

From the Departments of Molecular Biology and Physical Chemistry, the Hebrew University, Jerusalem, Israel.

α-BUNGAROTOXIN LABELING AND ACETYLCHOLINESTERASE LOCALIZATION AT THE MAUTHNER FIBER GIANT SYNAPSE IN THE HATCHETFISH

In the medulla of the South American hatchetfish, the Mauthner fiber forms large chemical synapses on a number of large myelinated axons termed giant fibers. These axons mediate excitation of pectoral fin motoneurons bilaterally and in this fish bilat-

eral pectoral fin adduction is an important component of the Mauthner fiber-mediated escape reflex. For the study presented here, autoradiographic and histochemical techniques have been used to help characterize the pharmacology of transmission at the Mauthner fiber giant synapse of the hatchetfish. [^{125}I]α-Bungarotoxin was applied to hatchetfish medullae and a standard autoradiographic procedure was carried out on 3- to 4-μm sections of glutaraldehyde-fixed tissue. All Mauthner fiber giant synapses, as identified by light microscopic criteria, had closely associated silver grains. Labeling was blocked by *d*-tubocurarine. Glutaraldehyde-fixed slices of hatchetfish medulla were stained histochemically for acetylcholinesterase; all giant synapses that could be identified in the light microscope showed heavy deposits of reaction product. Staining was blocked by diisopropylfluorophosphate, which inhibits both pseudocholinesterase and acetylcholinesterase, but was not blocked by tetraisopropylpyrophosphoramide, a specific pseudocholinesterase inhibitor. This evidence strongly supports the suggestion that the Mauthner fiber synapse is nicotinic cholinergic.

Day, J. W., Hall, D. H., *Hall, L. M.*, and Bennett, M. V. L.

The Journal of Neuroscience 3(2):272-279, 1983.

Other support: National Science Foundation and the National Institutes of Health.

From the Division of Cellular Neurobiology, Department of Neuroscience, Albert Einstein College of Medicine, the Bronx, NY.

RECENT DEVELOPMENTS IN INHALATION TOXICOLOGY: EVALUATION OF SELECTED SHORT-TERM ENDPOINTS FOLLOWING 'NOSE-ONLY' EXPOSURE OF RODENTS

Inhalation toxicology has routinely employed traditional inhalation chamber exposures, which can result in a distribution pattern where only 10-30% of the particulates are deposited in the lungs of the exposed animals. A major portion of the material is deposited on the hides of the animals and as a result of preening, 60-80% of the material is eventually found in the gastrointestinal tract. A need for alternative exposure systems led to the development of a "nose-only" exposure system where the animal is restrained and the nose of each animal protrudes into an exposure chamber. Under such conditions, the dose to the lung can be optimized, controlled and documented. Employing a "nose-only" inhalation system and using one particular environmental aerosol, cigarette smoke, a battery of short-term endpoints was applied to assess the biological activity of the material. Three assays are discussed in this paper: a pharmacokinetics assay, a cellular proliferation assay and a cytogenetics assay. These assays demonstrated that exposure to cigarette smoke could result in certain smoke components (*e.g.*, dotriacontane) exerting effects directly in the lung, as reflected by the cellular proliferation assay in lung tissue, and other chemicals (*e.g.*, catechol) exerting effects systemically, as reflected by the cytogenetics assay in bone marrow.

Henry, C. J. *et al. (Microbiological Associates, Inc.)*

Other support: In: Homburger, F. (ed.): *Safety Evaluation and Regulation of Chemicals*, Basel: S. Karger, 1983, pp. 233-242.

From the Division of Toxicology and Oncology, Microbiological Associates, Inc., Bethesda, MD.

SEROTONIN-INDUCED DISRUPTIC
I. SERUM PROGESTERONE, IMPL/ AND INTRAUTERINE pO$_2$

The experiments described in this p disruption of implantation in the rat is as and/or uterine vascular function. The serotonin, administered on the day after nates pregnancy in the rat. Consequent terone levels, implantation site blood flc to see whether the disruption of implant uterine vascular function. Animals rece saline (C: control) or serotonin (S: 20 mg alter the number of blastocysts implant resorption. Progesterone levels in sero controls at 6 hrs. postinjection or on Da blood flow was reduced at 30 min. and injection. A prompt and sustained redu nied the reduced uterine perfusion. Thu luteum function but is associated with blood flow and intraluminal oxygen ava tion in the rat are compared with those

Mitchell, J. A. and Hammer, R. E.

Biology of Reproduction 28:830-835, 1

From the Department of Anatomy, W Detroit.

SEROTONIN-INDUCED DISRUPTIC
II. SUPPRESSION OF DECIDUALIZ

This study was undertaken to dete disrupt implantation in the rat, also sup of serotonin on decidualization, luteal f pseudopregnant rats; effects on serum p tation were determined in pregnant ani capable of disrupting implantation in p pseudopregnant animals. Further, the achieved by administration of serotonin serotonin-treated pseudopregnant ra pregnancy was not altered, and an injec suppress plasma progesterone levels ir suppression of decidualization is not a Rather, impaired decidualization proba uterine blood flow and possibly sustaine tion. The effects of serotonin on decidu

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Bungarotoxin was applied to
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National Institutes of Health.

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genetics assay in bone marrow.

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SEROTONIN-INDUCED DISRUPTION OF IMPLANTATION IN THE RAT: I. SERUM PROGESTERONE, IMPLANTATION SITE BLOOD FLOW, AND INTRAUTERINE pO_2

The experiments described in this paper were undertaken to determine if serotonin disruption of implantation in the rat is associated with alterations in progesterone levels and/or uterine vascular function. The results of the first experiment showed that serotonin, administered on the day after the initiation of implantation, promptly terminates pregnancy in the rat. Consequently, the effects of serotonin on serum progesterone levels, implantation site blood flow and intrauterine O_2 tension were determined to see whether the disruption of implantation is related to altered corpus luteum and/or uterine vascular function. Animals received a subcutaneous injection of physiological saline (C: control) or serotonin (S: 20 mg/kg) on Day 5 of pregnancy. Serotonin did not alter the number of blastocysts implanting but did cause subsequent implantation site resorption. Progesterone levels in serotonin-treated rats did not differ from those of controls at 6 hrs. postinjection or on Days 6 through 10 of pregnancy. Implantation site blood flow was reduced at 30 min. and remained suppressed at 2 hrs. after serotonin injection. A prompt and sustained reduction in intrauterine oxygen tension accompanied the reduced uterine perfusion. Thus, disruption is not a result of impaired corpus luteum function but is associated with marked and protracted reductions in uterine blood flow and intraluminal oxygen availability. The effects of serotonin on implantation in the rat are compared with those of nicotine.

Mitchell, J. A. and Hammer, R. E.

Biology of Reproduction 28:830-835, 1983.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

SEROTONIN-INDUCED DISRUPTION OF IMPLANTATION IN THE RAT: II. SUPPRESSION OF DECIDUALIZATION

This study was undertaken to determine whether serotonin, at a dose known to disrupt implantation in the rat, also suppresses decidualization. To do this, the effects of serotonin on decidualization, luteal function or uterine blood flow were assessed in pseudopregnant rats; effects on serum progesterone levels and maintenance of implantation were determined in pregnant animals. Results indicate that doses of serotonin capable of disrupting implantation in pregnant rats also suppress decidualization in pseudopregnant animals. Further, the impairment of both phenomena is maximally achieved by administration of serotonin on Day 5. It is inferred that progesterone levels in serotonin-treated pseudopregnant rats are normal since the duration of pseudopregnancy was not altered, and an injection of an equivalent dose of serotonin did not suppress plasma progesterone levels in pregnant rats. Therefore, serotonin-induced suppression of decidualization is not a consequence of compromised luteal activity. Rather, impaired decidualization probably results from ischemia initiated by reduced uterine blood flow and possibly sustained and intensified by subsequent emboli formation. The effects of serotonin on decidualization are compared with those of nicotine.

Mitchell, J. A., Hammer, R. E. and Goldman, H.

Biology of Reproduction 29:151-156, 1983.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

CONCOMITANT REDUCTION IN UTERINE BLOOD FLOW AND INTRAUTERINE OXYGEN TENSION IN THE RAT FOLLOWING NICOTINE ADMINISTRATION

This study represents an attempt to determine the relationship between uterine blood flow and the availability of oxygen within the uterus and the effects of nicotine on such vascular-dependent phenomena as blood flow and capillary permeability at the implantation site. Pregnant Sprague-Dawley rats received an injection at 1200 h, day 5; pseudopregnant animals were injected between 1300 and 1400 h on day 4. Controls received a sc injection of an equivalent volume of saline. Measurements were then made of intrauterine oxygen, blood flow in whole uteri and at implantation sites, and of endometrical vascular permeability. Results show that nicotine suppresses whole uterine blood flow and luminal oxygen concentration, decreases perfusion at the implantation site and retards growth of the nidus. Whereas prior to implantation the well being of the free-living conceptus is dependent on the diffusion of blood-bones metabolic substrates from the endometrial vasculature to the uterine lumen; following nidation, maintenance of optimal conditions for continuation of pregnancy is dependent on metabolic exchange between the embryo and its implantation chamber. Nicotine, being capable of altering uterine vascular function both before and after uterine-blastocyst contact, exerts diverse effects on the course of pregnancy in the rat. The decreased concentration of oxygen and other metabolic substrates consequent to nicotine-induced reduction in uterine blood flow probably contributes to such preimplantation phenomena as reduced conceptus growth, delayed implantation and suppressed decidualization. Whereas once implantation is underway, the alkaloid retards embryo and/or nidus growth as indicated by reduced implantation site weight.

Mitchell, J. A., Hammer, R. E. and Goldman, H.

In: Bicher, H. I. and Bruley, D. F. (eds): *Oxygen Transport to Tissue – IV*, New York: Plenum Press Corporation, 1983, pp. 231-241.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

EFFECT OF GTP ANALOGUES ON PURIFIED SOLUBLE GUANYLATE CYCLASE

Although soluble guanylate cyclase from several mammalian sources has been purified recently, no information is available as of now on the stoichiometry of substrate binding or on the requirements of the nucleotide binding site. In this attempt to gain insight into these questions, as well as to understand more clearly the complex kinetic characteristics of guanylate cyclase, the effects of various nucleotide analogues on the enzyme were examined. Three GTP analogues, 5-guanylylimidodiphosphate (GMP-P(NH)P), guanylyl-(β,γ-methylene) diphosphate (GMP-(CH_2)P), and guanosine 5′-*O*-(3-thiotriphosphate) (GT
cyclase. GTPγS suported cyclic G
Mn^{2+}-GTP and Mg^{2+}-GTP, respecti
cyclic GMP formation at 10-20% c
analogues were found to have mult
sine triphosphate, and the 2′3′-dialc
for the enzyme; however, they we
logues could be useful tools for the s
they may also help elucidate the eff
cyclase regulation.

Brandwein, H. J., Lewicki, J. A.,

The Journal of Biological Chemist

Other support: National Institutes

From the Departments of Medicine
Veterans Administration Medical C

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WITHIN NEURONS OF RAT BR

The immunohistochemical loc
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by using four different monoclonal
lung enzyme. Immunofluorescence
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within medium spiny neurons of
neocortex. Cerebellar guanylate cy
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was abolished when the monoclona
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primary antibody. Immunohistoch
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clic GMP-dependent protein kinas
role for cyclic GMP in neurotransr

Ariano, M. A., Lewicki, J. A., Br

Proceedings of the National Acad
79:1316-1320, 1982.

Other support: Biomedical Resea
College of Medicine, and the Nati

From the Department of Anatomy
of Medicine, Burlington, and the D
ford University, Palo Alto Veteran

Wayne State University

LOW AND
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ıtionship between uterine
d the effects of nicotine on
ɔillary permeability at the
ı injection at 1200 h, day 5;
400 h on day 4. Controls
Measurements were then
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ɪ to Tissue–IV, New York:

, Wayne State University

BLE GUANYLATE

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nding site. In this attempt to
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various nucleotide analogues
5-guanylylimidodiphosphate
(GMP-(CH_2)P), and guano-

sine 5′-*O*-(3-thiotriphosphate) (GTPγS) were found to be substrates for guanylate cyclase. GTPγS suported cyclic GMP formation at 20 or 75% of the rate seen with Mn^{2+}-GTP and Mg^{2+}-GTP, respectively. GMP-P(NH)P and GMP-P(CH_2)P supported cyclic GMP formation at 10-20% of the GTP rate with either cation cofactor. These analogues were found to have multiple K_m values. Guanosine tetraphosphate, adenosine triphosphate, and the 2′3′-dialdehyde derivative of GTP were not good substrates for the enzyme; however, they were potent competitive inhibitors. These GTP analogues could be useful tools for the study of GTP binding sites on guanylate cyclase and they may also help elucidate the effects of free radicals and other agents on guanylate cyclase regulation.

Brandwein, H. J., Lewicki, J. A., Waldman, S. A., and *Murad, F.*

The Journal of Biological Chemistry 257(3):1309-1311, 1982.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

IMMUNOHISTOCHEMICAL LOCALIZATION OF GUANYLATE CYCLASE WITHIN NEURONS OF RAT BRAIN

The immunohistochemical localization of guanylate cyclase has been described in three central nervous system areas (rat neocortex, caudate-putamen and cerebellum) by using four different monoclonal antibodies directed against the soluble form of rat lung enzyme. Immunofluorescence could be seen within somata and proximal dendrites of neurons in these regions. A nuclear immunofluorescence reaction to guanylate cyclase was characteristically absent. The staining pattern for guanylate cyclase was coincident with previously described localizations of cyclic GMP immunofluorescence within medium spiny neurons of the caudate-putamen and pyramidal cells of the neocortex. Cerebellar guanylate cyclase immunoreactivity was primarily confined to Purkinje cells and their primary dendrites, similar to the pattern reported for cyclic GMP-dependent protein kinase localization. Guanylate cyclase immunofluorescence was abolished when the monoclonal antibodies were exposed to purified enzyme prior to incubation of the tissue slices or when control antibody was substituted for the primary antibody. Immunohistochemical localization of cyclic AMP in these same tissues was readily distinguished from that of guanylate cyclase or cyclic GMP, showing uniform fluorescence throughout the cell bodies of neurons and glial elements. The predominantly neuronal location of cyclic GMP, cyclic GMP phosphodiesterase, cyclic GMP-dependent protein kinase, and guanylate cyclase discussed here suggests a role for cyclic GMP in neurotransmission.

Ariano, M. A., Lewicki, J. A., Brandwein, H. J., and *Murad, F.*

Proceedings of the National Academy of Sciences of the United States of America 79:1316-1320, 1982.

Other support: Biomedical Research Support Grant to the University of Vermont College of Medicine, and the National Science Foundation.

From the Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, and the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

PROPERTIES OF PURIFIED SOLUBLE GUANYLATE CYCLASE ACTIVATED BY NITRIC OXIDE AND SODIUM NITROPRUSSIDE

For the experiments presented here, highly purified rat lung soluble guanylate cyclase was activated with nitric oxide or sodium nitroprusside and the degree of activation varied with incubation conditions. With Mg^{2+} as the cation cofactor, about 2- to 8-fold activation was observed with nitric oxide or sodium nitroprusside alone. Markedly enhanced activation (20-40 fold) was observed when 1μM hemin was added to the enzyme prior to exposure to the activating agent. Activation with hemin and sodium nitroprusside was prevented in a dose-dependent manner by sodium cyanide. The level of activation was increased by the addition of 1 mM dithiothreitol, but unlike hemin which had no effect on basal enzyme activity, dithiothreitol led to a considerable increase in basal activity. In another study, of the reversibility of the activation of guanylate cyclase by nitric oxide and sodium nitroprusside, it was seen that activated guanylate cyclase decayed to basal activity within one hour at 2°C and the enzyme could be reactivated upon re-exposure to nitroprusside or nitric oxide. Under basal conditions, Michaelis-Menten kinetics were observed, with a K_m for GTP of 140 μM with Mg^{2+} cofactor. Following activation with nitroprusside or nitric oxide, curvilinear Eadie-Hofsteen transformations of kinetic data were observed, with K_m's of 22 μM and 100 μM for Mg-GTP. Similar curvilinear Eadie-Hofstee transformations were observed with Mn^{2+} as the cation cofactor. These and other data suggest that multiple GTP catalytic sites are present in activated guanylate cyclase or, alternatively, multiple populations of enzyme exist.

Lewicki, J. A., Brandwein, H. J., Mittal, C. K., Arnold, W. P., and *Murad, F.*

Journal of Cyclic Nucleotide Research 8(1):17-25, 1982.

Other support: National Institutes of Health and National Research Service Awards.

From the Departments of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA, and the Department of Anesthesiology, University of Virginia, Charlottesville.

SODIUM NITROPRUSSIDE-INDUCED PROTEIN PHOSPHORYLATION IN INTACT RAT AORTA IS MIMICKED BY 8-BROMO CYCLIC GMP

This pharmacological study with intact rat thoracic aorta demonstrates that nitroprusside can alter the incorporation of ^{32}P into proteins from soluble and particulate fractions of the aorta. Furthermore, the pattern of altered protein phosphorylation evaluated with two-dimensional gel electrophoresis was mimicked by 8-bromo cyclic GMP. Approximately 100- and 10-fold greater concentrations of 8-bromo cyclic AMP and dibutyryl cyclic AMP, respectively, were required in order to increase ^{32}P incorporation into certain proteins whose phosphorylation was altered by 8-bromo cyclic GMP or nitroprusside. Isoproterenol also increased ^{32}P incorporation into some proteins: the incorporation of ^{32}P into some proteins was preferentially increased by dibutyryl cyclic AMP. Overall, the pattern of ^{32}P incorporation induced by a relatively high concentration of isoproterenol 0.1 mM was similar but not identical to that seen with 0.5 mM dibutyryl cyclic AMP. This study indicates that the incorporation of ^{32}P into endogenous proteins of intact rat aorta can be regulated by nitroprusside. These effects can be mimicked by cyclic GMP analogues and only partially by cyclic AMP analogues or isoproterenol. Presumably, these effects of nitroprusside are mediated through a cyclic

GMP-dependent process (protein ki
relaxant properties of nitroprusside

Rapoport, R. M., Draznin, M. B. a

Proceedings of the National Acad
79:6470-6474, 1982.

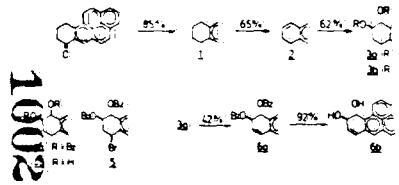
Other support: National Institutes o
Research Service Award.

From the Department of Medicine a
Veterans Administration Medical C

EFFICIENT SYNTHESIS OF NO
POLYCYCLIC AROMATIC HYD
CATECHOLS

Non-K-region dihydrodiols of
important role in the metabolism o
epoxides, some of which are consi
PAH. The two currently used meth
introduction of the *trans*-diol struc
reaction or the reduction of a suita
cumbersome and not too efficient. I
tion of non-K-region *o*-quinones w
only when the olefinic bond was fi
found that non-K-region *o*-quinone
sodium borohydride if the reaction
summarizes the results obtained wit
reduced with sodium borohydride.
chols, e.g., 3a, and their diacetates,
e.g., 1a, all three compounds being
reduced with sodium borohydride i

Scheme I



The results presented in this paper
atechols can be efficiently and co
borohydride, in the presence of oxyg
dihydrodiols has the additional adva
workup and thus competes favorabl

GMP-dependent process (protein kinase or phosphatase) which may play a role in the relaxant properties of nitroprusside and cyclic GMP.

Rapoport, R. M., Draznin, M. B. and *Murad, F.*

Proceedings of the National Academy of Sciences of the United States of America 79:6470-6474, 1982.

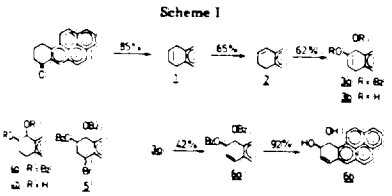
Other support: National Institutes of Health, Veterans Administration, and National Research Service Award.

From the Department of Medicine and Pharmacology, Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

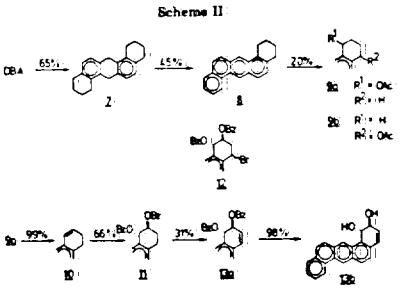
EFFICIENT SYNTHESIS OF NON-K-REGION *TRANS*-DIHYDRODIOLS OF POLYCYCLIC AROMATIC HYDROCARBONS FROM *O*-QUINONES AND CATECHOLS

Non-K-region dihydrodiols of polycyclic aromatic hydrocarbons (PAH) play an important role in the metabolism of PAH since they are precursors of dihydrodiol epoxides, some of which are considered to be ultimate carcinogenic metabolites of PAH. The two currently used methods for the synthesis of *trans*-dihydrodiols – the introduction of the *trans*-diol structure into a suitable dihydroarene by the Prévost reaction or the reduction of a suitable *o*-quinone with complex metal hydrides – are cumbersome and not too efficient. Following a different method, the efficient reduction of non-K-region *o*-quinones with sodium borohydride seemed possible before only when the olefinic bond was first converted to the dibromide. However, it was found that non-K-region *o*-quinones can be efficiently reduced to dihydrodiols with sodium borohydride if the reaction is performed in the presence of oxygen. Table I summarizes the results obtained with 1,2-dioxygenated derivatives of naphthalene are reduced with sodium borohydride. It should be emphasized that non-K-region catechols, e.g., 3a, and their diacetates, e.g., 5a, can conveniently replace the *o*-quinones, e.g., 1a, all three compounds being converted to the dihydrodiol, e.g., 4a, if they are reduced with sodium borohydride in the presence of oxygen.

Scheme I



Scheme II



The results presented in this paper demonstrate that non-K-region *o*-quinones and catechols can be efficiently and conveniently reduced to dihydrodiols with sodium borohydride, in the presence of oxygen. This method of preparing non-K-region *trans*-dihydrodiols has the additional advantage of a very simple experimental procedure and workup and thus competes favorably with the earlier methods of preparation.

Platt, K. L. and *Oesch, F.*

The Journal of Organic Chemistry 48:265-268, 1983.

From the Institute of Pharmacology, Section on Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

FREE RADICAL PATHOLOGY: INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY PRODUCTS FROM THE REACTION OF NITROGEN DIOXIDE WITH HYDROGEN PEROXIDE AND THE ETIOLOGY OF EMPHYSEMA

This paper presents a study of the production of biologically damaging species from the reaction of nitrogen dioxide with hydrogen peroxide using the oxidative inactivation of human α-1-proteinase inhibitor as a model reaction. In this work, exposure of human α-1-proteinase inhibitor to 75 ppm nitrogen dioxide (NO_2) and 1 mM hydrogen peroxide (H_2O_2) resulted in a 65% loss in the anti-elastase activity of the protein after one hour. Exposure to either NO_2 or H_2O_2 alone had no effect. The inhibition was partially prevented when mannitol or superoxide dismutase was included during the exposure, implying that superoxide and the hydroxyl radical are formed in reactions between NO_2 and H_2O_2 and play a role in the inhibitory process. In conclusion, in this paper the authors have demonstrated the inactivation of the anti-elastase activity of human α-1-proteinase by species produced from the reaction of NO_2 with H_2O_2. The inhibition of this inactivation by mannitol and superoxide dismutase implies that these reactive species include superoxide and the hydroxyl radical, and may, upon extrapolation of the data, react directly with elastin and other lung tissue in direct damage-producing reactions.

Dooley, M. M. and *Pryor, W. A.*

Biochemical and Biophysical Research Communications 106(3):981-987, 1982.

Other support: National Institutes of Health and the National Science Foundation.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

ELECTRON-SPIN RESONANCE STUDY OF MAINSTREAM AND SIDESTREAM CIGARETTE SMOKE: NATURE OF THE FREE RADICALS IN GAS-PHASE SMOKE AND IN CIGARETTE TAR

In this chemical study, radicals in the gas phase of both mainstream and sidestream cigarette smoke have been monitored using electron-spin resonance (ESR) spin-trapping techniques with α-phenyl-N-*tert*-butyl nitrone (PBN) as the spin-trap. The principal radicals trapped in this way appear to be alkoxyl radicals. Mainstream and sidestream gas-phase smoke each has about the same concentration of radicals, about 1×10^{16} radicals per cigarette (or 5×10^{14} per puff). These radicals are reactive, yet they appear to be remarkably long-lived since they are still spin-trapped from gas-phase smoke after more than five min. The authors propose that a steady-state concentration of reactive radicals exists in gas phase cigarette smoke. They suggest that this steady state is produced by the slow oxidation of nitric oxide (present in high concentrations in smoke and relatively unreactive) to the more reactive nitrogen dioxide, followed by the reaction of nitrogen dioxide with reactive organic molecules in smoke (such as olefins and dienes). Preliminary experiments reported here support this hy-

pothesis. It is also shown in this pap
smoke contains persistent free radical
g values of 2.003. The tar radical c
organic solvents, and a variety of fra
solutions. Most of the radicals occur
leaf pigments. Treatment of alcoholic
of radicals that appear to be semiqui
phenolic and polyphenolic species in

Pryor, W. A., Prier, D. G. and Chur

Environmental Health Perspectives 4

Other support: National Institutes of

From the Department of Chemistry,

REACTIVE OXY-RADICALS FRO PHYSIOLOGICAL EFFECTS

Cigarette smoke contains high cc
in tar. In this paper, the nature of the
and it is suggested that radical-mediat
logical effects of smoke. Sections
RADICAL, THE GAS PHASE SMC
SMOKE, CELLULOSE "CIGARET
and ADDITIONAL RADICAL MEC
TEASE. In summary, it seems that al
pulmonary damage. Alkoxyl radicals
nary target molecules such as unsatu
proteins. Smoke also stimulates pulm
addition, NO_2 can react with the hy
radicals and/or superoxide. Finally, t
the tar radical) might cause the oxidat
smoke to carcinogenic species.

Pryor, W. A., et al.

In: Greenwald/Cohen (eds.): *Oxy Radi*
Medical Aspects, New York: Elsevier

Other support: National Science Fou
National Foundation for Cancer Rese

From the Department of Chemistry, L

ENKEPHALIN- AND SUBSTANCE MAMMALIAN SPERM AND ACCE

For the study presented here, enk
stance P were extracted from human, b
and accessory sex glands of the rat;
and the biological activity of peptides
higher levels of methionine enkephali

pothesis. It is also shown in this paper that tar from both mainstream and sidestream smoke contains persistent free radicals that exhibit broad, single-line ESR spectra with *g* values of 2.003. The tar radical can be extracted into *tert*-butylbenzene and other organic solvents, and a variety of fractionation procedures have been applied to these solutions. Most of the radicals occur in the fractions that contain the phenolic tobacco leaf pigments. Treatment of alcoholic solutions of tar with base generates a new group of radicals that appear to be semiquinone radicals derived from the oxidation of the phenolic and polyphenolic species in tar.

Pryor, W. A., Prier, D. G. and Church, D. F.

Environmental Health Perspectives 47:345-355, 1983.

Other support: National Institutes of Health.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

REACTIVE OXY-RADICALS FROM CIGARETTE SMOKE AND THEIR PHYSIOLOGICAL EFFECTS

Cigarette smoke contains high concentrations of radicals both in the gas phase and in tar. In this paper, the nature of the radicals in tar and in the gas phase are discussed and it is suggested that radical-mediated mechanisms account for some of the physiological effects of smoke. Sections of the paper are devoted to THE TAR FREE RADICAL, THE GAS PHASE SMOKE FREE RADICAL, NO_x CHEMISTRY IN SMOKE, CELLULOSE "CIGARETTE" AND EXCITED SPECIES IN SMOKE, and ADDITIONAL RADICAL MECHANISMS–DESTRUCTION OF ANTIPROTEASE. In summary, it seems that all of the radical-producing processes might cause pulmonary damage. Alkoxyl radicals in smoke could directly attack reactive pulmonary target molecules such as unsaturated lipids or sulfur-containing enzymes and proteins. Smoke also stimulates pulmonary macrophages to produce O_2^- and H_2O_2. In addition, NO_2 can react with the hydrogen peroxide produced to release hydroxyl radicals and/or superoxide. Finally, the radicals in smoke (peroxyl, alkoxyl, NO_2, or the tar radical) might cause the oxidation of the polycyclic aromatic hydrocarbons in smoke to carcinogenic species.

Pryor, W. A., et al.

In: Greenwald/Cohen (eds.): *Oxy Radicals and Their Scavenger Systems: Cellular and Medical Aspects*, New York: Elsevier, 1983, vol. II, pp. 185-192.

Other support: National Science Foundation, National Institutes of Health and the National Foundation for Cancer Research.

From the Department of Chemistry, Louisiana State University, Baton Rouge.

ENKEPHALIN- AND SUBSTANCE P-LIKE IMMUNOREACTIVITIES OF MAMMALIAN SPERM AND ACCESSORY SEX GLANDS

For the study presented here, enkephalins and related opioid peptides, and Substance P were extracted from human, bull and rat spermatozoa, human seminal plasma and accessory sex glands of the rat; radioimmunoassays were run on these extracts and the biological activity of peptides was characterized. Results showed there were higher levels of methionine enkephalin and leucine enkephalin in seminal plasma than

in spermatozoa and higher levels of Substance P were present in sperm cells than in plasma. Specifically, the extracts of spermatozoa, seminal plasma and accessory sex glands produced the following responses: (1) Extracts containing very high immunoreactivities to opioid peptides compared to Substance P (*e.g.*, extract of human seminal plasma) inhibited chemical transmission. (2) Extracts containing very high immunoreactivities to Substance P compared to the opioid peptide (*e.g.*, caudal epididymis) facilitated transmission. (3) All other extracts caused biphasic responses. Overall, these results indicate methionine enkephalin and Substance P may play a regulatory role in acetylcholine-induced Ca^{2+} movements and, therefore, progressive sperm motility.

Sastry, B. V. R. et al.

Biochemical Pharmacology 31(21):3519-3522, 1982.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

CHANGES IN ENZYMES OF THE CHOLINERGIC SYSTEM AND ACETYLCHOLINE RELEASE IN THE CEREBRA OF AGING MALE FISCHER RATS

Since the functional decline of memory in the aging human brain has been partially attributed to defects in cholinergic transmission, it was decided to investigate various components of the cholinergic system in cerebra of Fischer 344 male rats, ages 3-33 months. Choline acetyltransferase (ChA), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) activities were determined in homogenates of the cerebra using specific radiometric assays. For measuring the release of acetylcholine (ACh), cerebral slices were incubated for one hour in Krebs buffer containing ^{3}H-choline chloride to label ACh formed *in situ*, washed, and transferred to a microbath for superfusion. ^{3}H-ACh released into the superfusate was determined. Results showed that the levels of ChA in the cerebra of 9- to 27-month-old rats were lower than those in 3-month-old rats. Only 1% of these rats survive to the age of 33 months, while in rats of this age there was no decrease in ChA levels. The survival of a small group of Fischer 344 rats up to 33 months and the maintenance of ChA activities in this group suggests the possibility of an interaction of age and genetic factors in the control of cortical cholinergic mechanisms. In addition, AChE decreased while BChE increased with advancing age. The rate of spontaneous release of ^{3}H-ACh decreased gradually by 63% from 3 to 33 months of age. The evoked release of ACh decreased by 50% in 33-month-old rats. Alterations in the levels of ChA, AChE (or BChE) and cholinergic receptors are not large enough to account for losses in cholinergic transmission in the cerebrum. The large drops in the rates of spontaneous or evoked release of ACh in the aging cerebrum indicate that the functional defect in the cholinergic transmission of the aging cerebrum is possibly due to a defective release mechanism of this transmitter.

Sastry, B. V. R. et al.

Pharmacology 26:61-72, 1983.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

DEPRESSION OF HUMAN SPERM ENZYMATIC METHYLATION

This report describes, for the firs
ance to motility of phospholipid N-m
In this work, enzymatic phospholipic
consequences of their inhibition on n
date here suggest that there are at least
first enzyme, PMT I, converted mem
phosphatidyl-N-methylethanolamine
of 8.0. The second PMT converted PM
and PC. It had a K_m of 71 μM and a pH
protein carboxymethylase (PCM) and
lar levels of S-adenosylhomocysteine
mediated methylations, were increase
tone (L-HCT), and erythro-9-(2-hydr
adenosine deaminase, to human sperm
suspension was determined every hou
adenosine, L-HCT and EHNA, the
similar conditions, phospholipid meth
ments were also conducted in the prese
in the inhibition of phospholipid methy
homogenates had the following order
indicate that the PMT system and/or t
the regulation of human sperm motilit

Sastry, B. V. R. and Janson, V. E.

Biochemical Pharmacology 32(8):142

Other support: National Institute of
National Institute of Aging.

From the Department of Pharmacolo
Nashville, TN.

REACTION OF PEPTIDE THIOBEN CHYMOTRYPSINLIKE ENZYMES

Several chymotrypsinlike serine p
turnover of various proteins, have re
include rat mast cell protease I, rat mas
enzyme, dog skin chymotrypsinlike e
vine chymotrypsin A. In this attempt t
these enzymes, kinetic constants as we
of the peptide substrates succinyl-phen
and succinyl-alanyl-alanyl-prolyl-phe

sent in sperm cells than in
plasma and accessory sex
aining very high immuno-
., extract of human seminal
aining very high immuno-
(*e.g.*, caudal epididymis)
ic responses. Overall, these
nay play a regulatory role
progressive sperm motility.

ersity School of Medicine,

'STEM AND
AGING MALE FISCHER

human brain has been par-
was decided to investigate
Fischer 344 male rats, ages
inesterase (AChE), and bu-
homogenates of the cerebra
ase of acetylcholine (ACh),
uffer containing ^{3}H-choline
nsferred to a microbath for
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rats were lower than those in
of 33 months, while in rats of
l of a small group of Fischer
ivities in this group suggests
rs in the control of cortical
while BChE increased with
decreased gradually by 63%
h decreased by 50% in 33-
(or BChE) and cholinergic
olinergic transmission in the
evoked release of ACh in the
olinergic transmission of the
chanism of this transmitter.

iversity School of Medicine,

DEPRESSION OF HUMAN SPERM MOTILITY BY INHIBITION OF ENZYMATIC METHYLATION

This report describes, for the first time, the occurrence, properties and significance to motility of phospholipid N-methyltransferase (PMT) in human spermatozoa. In this work, enzymatic phospholipid methylation and carboxymethylation, and the consequences of their inhibition on motility, were studied using human sperm. The date here suggest that there are at least two PMT enzymes in human spermatozoa. The first enzyme, PMT I, converted membrane bound phosphatidylethanolamine (PE) to phosphatidyl-N-methylethanolamine (PME). It had a K_m of 4.0 μM and a pH optimum of 8.0. The second PMT converted PME to phosphatidyl-N,N-dimethylethanolamine and PC. It had a K_m of 71 μM and a pH optimum of 10.0. Spermatozoa also contained protein carboxymethylase (PCM) and methyl acceptor protein (MAP). The intracellular levels of S-adenosylhomocysteine (SAH), an inhibitor of S-adenosylmethionine-mediated methylations, were increased by adding adenosine, L-homocysteine thiolactone (L-HCT), and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), an inhibitor of adenosine deaminase, to human sperm ejaculates. The motility index of each sperm suspension was determined every hour for four hrs. In the presence of the mixture of adenosine, L-HCT and EHNA, the motility index was depressed by 57%. Under similar conditions, phospholipid methylation was depressed by 48%. Similar experiments were also conducted in the presence of 3-deazaadenosine. The potencies of SAH in the inhibition of phospholipid methylation and protein carboxymethylation in sperm homogenates had the following order: PMT I>PCM>PMT II. These observations indicate that the PMT system and/or the PCM-MAP system play a significant role in the regulation of human sperm motility.

Sastry, B. V. R. and Janson, V. E.

Biochemical Pharmacology 32(8):1423-1432, 1983.

Other support: National Institute of Child Health and Human Development and the National Institute of Aging.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REACTION OF PEPTIDE THIOBENZYL ESTERS WITH MAMMALIAN CHYMOTRYPSINLIKE ENZYMES: A SENSITIVE ASSAY METHOD

Several chymotrypsinlike serine proteases, which are thought to play a role in the turnover of various proteins, have recently been isolated and characterized. These include rat mast cell protease I, rat mast cell protease II, human skin chymotrypsinlike enzyme, dog skin chymotrypsinlike enzyme, human leukocyte cathepsin G, and bovine chymotrypsin A. In this attempt to develop a sensitive method for the detection of these enzymes, kinetic constants as well as the maximum sensitivity for the hydrolysis of the peptide substrates succinyl-phenyl-alanyl-leucyl-phenylalanine thiobenzyl ester and succinyl-alanyl-alanyl-prolyl-phenylalanine thiobenzyl ester were determined.

Hydrolysis rates were followed spectrophotometrically at 324 nm by the formation of 4-thiopyridone, the product of the reaction between benzylthiol, released during hydrolysis of the peptide thiobenzyl esters, and 4,4′-dithiodipyridine present in the assay mixture. Peptide thiobenzyl ester substrates were shown to be very sensitive substrates, predominantly because of the large extinction coefficient of 4-thiopyridine and the high k_{cat}/K_m values for these compounds. Overall, the assays reported here are more sensitive than other methods previously reported and it seems that these thioester substrates could be extremely useful in future studies of the physiological role of these mammalian chymotrypsinlike enzymes.

Harper, J. W., Ramirez, G. and Powers, J. C. *(Travis, J.)*

Analytical Biochemistry 118:382-387, 1981.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

EFFECT OF CIGARETTE SMOKING ON BASAL AND CARBON DIOXIDE STIMULATED CEREBRAL BLOOD FLOW IN MAN

This study was undertaken to determine whether smoking is paralleled by a decreased, increased or unaffected cerebral blood flow (CBF). Healthy habitual cigarette smokers, ten men and three women, aged 21-48 years, were tested for the effect of smoking on CBF and on the cerebral hyperemia induced by CO_2 breathing. CBF was measured with the N_2O-wash-in technique in the basal state and during inhalation of 5% CO_2, before and after smoking of two commercial filter tipped cigarettes. In parallel, the (arterial-jugular venous) difference in O_2 content, arterial and jugular venous $_pCO_2$, pulmonary ventilation, heart rate and systemic blood pressure were followed. Results showed that during smoking there was a 10-15 mm Hg increase in systemic blood pressure and a parallel elevation of heart rate (+20 beats/min). CBF increased by about 25%, and cerebral vascular resistance fell about 15%. The cerebral metabolic rate of oxygen ($CMRO_2$) was elevated by about 30% above control. Inhalation of 5% CO_2 by itself markedly increased CBF and decreased cerebral vascular resistance, while leaving $CMRO_2$ unaffected. Cigarette smoking did not significantly change either of these effects of CO_2 breathing. Overall, from these data it is concluded that cigarette smoking elevates systemic blood pressure and decreases cerebral vascular resistance, and thereby augments basal CBF. This flow-promoting effect of smoking is probably due to an increased cerebral consumption of oxygen. Furthermore, the data demonstrate that smoking does not interfere with the cerebral vascular response to increased arterial $_pCO_2$.

Wennmalm, Å.

Clinical Physiology 2(6):529-535, 1982.

Other support: The Swedish Tobacco Company.

From the Department of Clinical Physiology, Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

NICOTINE INHIBITS VASCULAR THROMBOXANE FORMATION

The metabolism of arachidonic involves formation of a number of p taglandins (PG), thromboxane (Tx), paper, the metabolism of AA in two lium – is discussed with special ref metabolism. The effect of nicotine c from human platelets, while the effe was studied in rings of rabbit aorta presence of nicotine or indometha [^{14}C]arachidonic acid to [^{14}C]thromb unaffected by nicotine, (2) nicotine like activity formed in incubations cigarette smoking markedly inhibits human forearm. On the basis of thes vascular prostacyclin but not on p action by nicotine may facilitate pl reactive hyperemia. The data demo hyperemia in human skeletal mus reactive hyperemia, probably acts v inhibition of PG formation.

Wennmalm, Å. and Alster, P.

General Pharmacology 14(1):189-19

Other support: Swedish Tobacco C

From the Department of Clinical P Huddinge University Hospital, Hud

NICOTINE INHIBITS PROSTAG MICROSOMES

For this study of the influenc microsomal fractions of human ki patients, three men and three wom samples taken from the renal cortex and cortical and medullary microsc the absence and presence of nicotin methacin and with heat-inactivate diochromatograms of the renal co always displayed three major, and $PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. In additio some chromatograms of microsoma prostacyclin is the principal PG pro nicotine to the incubates resulted

NICOTINE INHIBITS VASCULAR PROSTACYCLIN BUT NOT PLATELET THROMBOXANE FORMATION

The metabolism of arachidonic acid (AA), a poly-unsaturated C:20 fatty acid, involves formation of a number of potentially active agents such as the primary prostaglandins (PG), thromboxane (Tx), prostacyclin (PGI_2), and the leukotrienes. In this paper, the metabolism of AA in two different tissues – platelets and vascular endothelium – is discussed with special reference to the effect elicited by nicotine on such metabolism. The effect of nicotine on the formation of Tx was studied in microsomes from human platelets, while the effect of nicotine on the formation of PGI_2-like activity was studied in rings of rabbit aorta incubated at room temperature in the absence or presence of nicotine or indomethacin. Results showed that (1) the conversion of [^{14}C]arachidonic acid to [^{14}C]thromboxane B_2 in rabbit or human platelet microsomes is unaffected by nicotine, (2) nicotine dose-relatedly inhibits the amount of prostacyclin-like activity formed in incubations of rabbit aortic rings or human veins, and (3) cigarette smoking markedly inhibits prostaglandin dependent reactive hyperemia in the human forearm. On the basis of these observations, it is concluded that nicotine acts on vascular prostacyclin but not on platelet thromboxane formation. Such a selective action by nicotine may facilitate platelet aggregability and counteract PG-dependent reactive hyperemia. The data demonstrate that (a) cigarette smoking inhibits reactive hyperemia in human skeletal muscle, and (b) cigarette smoking, when inhibiting reactive hyperemia, probably acts via the same mechanism as indomethacin, *i.e.*, via inhibition of PG formation.

Wennmalm, Å. and Alster, P.

General Pharmacology 14(1):189-191, 1983.

Other support: Swedish Tobacco Company.

From the Department of Clinical Physiology, Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

NICOTINE INHIBITS PROSTAGLANDIN SYNTHESIS IN HUMAN KIDNEY MICROSOMES

For this study of the influence of nicotine on prostaglandin (PG) synthesis in microsomal fractions of human kidney, samples were obtained from six informed patients, three men and three women, aged 42-61, undergoing nephrectomy. Tissue samples taken from the renal cortex and renal medulla were minced and homogenized, and cortical and medullary microsomal fractions were prepared and incubated both in the absence and presence of nicotine. Control incubations were carried out with indomethacin and with heat-inactivated microsomal fractions. Results showed that radiochromatograms of the renal cortical and renal medullary microsomal incubates always displayed three major, and well-defined, radiopeaks in parallel with 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. In addition, a low peak corresponding to PGD_2 appeared in some chromatograms of microsomal incubates of both tissues. These results reveal that prostacyclin is the principal PG product formed in human renal tissue. The addition of nicotine to the incubates resulted in a dose-related decrease in the amount of the

labelled PGs formed, and this effect was similar in both the renal cortical and renal medullary microsomal incubates. Indomethacin also induced a dose-related inhibition of the synthesis of [^{14}C]-PGs. However, indomethacin was much more efficient as an inhibitor of the [^{14}C]-PG formation, its potency in this respect being about 1000 times higher than that of nicotine. The fact that nicotine did not affect the distribution of the [^{14}C]-PGs formed suggests that the drug acted at the cyclo-oxygenase level, which is a step in synthesis common to all PGs. The authors of this paper suggest that the observed inhibitory effect of nicotine, even though less pronounced than that of indomethacin, deserves attention since it may provide a linkage between cigarette smoking and its sequelae in the cardiovascular system.

Alster, P. *et al. (Wennmalm, Å.)*

Acta Physiologica Scandinavica 117(4):581-583, 1983.

Other support: Swedish Tobacco Company, Swedish Medical Research Council, Magnus Bergvall Foundation, and the Karolinska Institutet, Stockholm.

From the Karolinska Institutet, Departments of Clinical Physiology, Pathology and Surgery, Huddinge Hospital, Huddinge, Sweden, and Clinical Physiology, St. Erik's Hospital, Stockholm.

EFFECT OF NICOTINE ON PROSTACYCLIN FORMATION IN RAT AORTA

The aim of the current study was to characterize the effect of nicotine on the conversion of arachidonate to prostacyclin (PGI_2) in the rat aorta. To do this, the effect of nicotine on the formation of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a metabolite of PGI_2 in the rat aorta, was investigated. Slices of rat aorta were incubated with [^{14}C]arachidonic acid ([^{14}C]AA) or ^{14}C-labeled prostaglandin endoperoxide H_2([^{14}C]PGH_2) in the presence of nicotine or indomethacin. ^{14}C-labeled products formed during the incubations were separated and quantified using thin layer chromatography and liquid scintillation spectrometry. A time- and substrate concentration-dependent formation of [^{14}C]6-keto-$PGF_{1\alpha}$ from [^{14}C]AA was found in the incubations. The K_M of AA for the formation of 6-keto-$PGF_{1\alpha}$ was 1.3×10^{-5} M. Nicotine competitively inhibited the formation of [^{14}C]6-keto-$PGF_{1\alpha}$ from [^{14}C]AA, the I_{50} being about 2×10^{-4} M. Indomethacin also decreased the formation of [^{14}C]keto-$PGF_{1\alpha}$ from [^{14}C]AA, with an I_{50} of about 10^{-6} M. Incubation of rat aorta slices with [^{14}C]PGH_2 also elicited the formation of [^{14}C]keto-$PGF_{1\alpha}$. This conversion was not affected by nicotine. It is concluded that nicotine inhibits the formation of PGI_2 in the rat aorta by competitive inhibition of the cyclo-oxygenase that converts arachidonate to prostaglandin endoperoxide. Furthermore, the data demonstrate that nicotine does not affect PGI_2 synthetase in this tissue.

Alster, P. and *Wennmalm, Å.*

European Journal of Pharmacology 86:441-446, 1983.

Other support: Swedish Tobacco Company and the Swedish Medical Council.

From the Department of Clinical Physiology at Karolinska Institutet, Stockholm, and Huddinge University Hospital, Huddinge, Sweden.

VI. *Immunolo*

INHIBITION OF INTERFER(SUPPRESSOR (SIRS) MEDI/

Two reasonably well defi reported here; immunosuppres (IFNβ) or soluble immune resp activated murine T cells. SIRS SIRS, release a second factor, responsible for suppression. N peroxide can convert SIRS to M which produce a factor(s) with immune responses *in vivo* and i in clinically depressed hosts. T suppression were examined to g of this agent. SIRS or IFNβ su control responses: under optima of control levels in the presenc SIRS or Mø-SF directly, but inl conversion of SIRS to Mø-SF b sole inhibits SIRS- or IFNβ-me and suggest that some immunoc for by interference with nonspe

Aune, T. M. and Pierce, C. W.

International Journal of Immun

Other support: U. S. Public He

From the Department of Patholo St. Louis, and the Department Washington University School

MY-1, THE HUMAN MYELOI MONOCLONAL ANTIBODIE LACTO-*N*-FUCOPENTAOSE I

The anti-My-1 monoclonal mouse immunized with HL60 hu locytes and granulocytic precurs cytes, monocytes, platelets, or detected by this monoclonal antil cytic precursor cells. The sugar

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Medical Council.

titutet, Stockholm, and

VI. *Immunology and Adaptive Mechanisms*

INHIBITION OF INTERFERON OR SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) MEDIATED SUPPRESSION BY LEVAMISOLE

Two reasonably well defined suppressor systems were employed in the studies reported here; immunosuppression was achieved with murine fibroblast interferon (IFNβ) or soluble immune response suppressor (SIRS), a product of concanavalin A-activated murine T cells. SIRS acts via macrophages (Mø) which after exposure to SIRS, release a second factor, Mø-derived suppressor factor (Mø-SF), that is directly responsible for suppression. Mø-SF appears to be modified SIRS since hydrogen peroxide can convert SIRS to Mø-SF directly. IFNβ also activates suppressor T cells which produce a factor(s) with properties identical to SIRS. Levamisole enhances immune responses *in vivo* and *in vitro* and is of value in improving immune responses in clinically depressed hosts. The effects of levamisole on IFNβ- or SIRS-mediated suppression were examined to gain a better understanding of the mechanism of action of this agent. SIRS or IFNβ suppressed plaque forming cell responses to 10-20% of control responses: under optimal conditions, these responses were increased to ~ 80% of control levels in the presence of levamisole. Levamisole did not inactivate IFNβ, SIRS or Mø-SF directly, but inhibited production of Mø-SF by SIRS-treated Mø and conversion of SIRS to Mø-SF by peroxide. These and other data indicate that levamisole inhibits SIRS- or IFNβ-mediated suppression by preventing formation of Mø-SF and suggest that some immunoenhancing properties of levamisole may be accounted for by interference with nonspecific suppressor T cell pathways.

Aune, T. M. and Pierce, C. W.

International Journal of Immunopharmacology 5(1):91-98, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology and Immunology, Washington University School of Medicine, St. Louis.

MY-1, THE HUMAN MYELOID-SPECIFIC ANTIGEN DETECTED BY MOUSE MONOCLONAL ANTIBODIES, IS A SUGAR SEQUENCE FOUND IN LACTO-*N*-FUCOPENTAOSE III

The anti-My-1 monoclonal antibody, produced by a hybridoma obtained from a mouse immunized with HL60 human promyelocytic leukemia cells, reacts with granulocytes and granulocytic precursor cells but not with normal peripheral blood lymphocytes, monocytes, platelets, or red cells. The cell surface antigen (designated My-1) detected by this monoclonal antibody is expressed on human granulocytes and granulocytic precursor cells. The sugar sequence

Galβ1-4GlcNAcβ1-3Gal....
3
|
Fucα1

which occurs in the glycolipid, lacto-*N*-fucopentaose III ceramide, in several higher

glycolipids, and in glycoproteins, contains the epitope recognized by anti-My-1 and 17 other monoclonal antibodies with the same cellular specificity. Although My-1 is strongly expressed only on granulocytic cells in the human hematopoietic system, it is also strongly expressed on some nonhematopoietic human and murine cells as well.

Huang, L. C., *Civin, C. I.*, Magnani, J. L., Shaper, J. H., and Ginsburg, V.

Blood 61(5):1020-1023, 1983.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; the Department of Pharmacology, University of Virginia, Charlottesville; and the Division of Pediatric Oncology, the Oncology Center, and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine, Baltimore.

STUDIES ON THE PATHOGENESIS OF THE ADULT RESPIRATORY DISTRESS SYNDROME

These studies of bronchoalveolar lavage (BAL) fluid from patients with adult respiratory distress syndrome (ARDS) were undertaken in an attempt to identify and characterize substances capable of activating components of the complement, contact, and clotting systems of plasma. Proteolytic activity of the fluids was observed by measuring cleavage of radiolabeled proteins of the contact (Hageman factor) and complement systems. Proteolytic activity was observed in 17 of 24 patients with ARDS, and BAL fluid of the seven ARDS patients without demonstrable, active enzyme exhibited inhibitory activity for the proteolytic activity. The enzymes cleaved Hageman factor, prekallikrein, plasminogen, high molecular weight kinogen, C4, C3, C5, and Factor B of the complement system. Cleavage of the contact system proteins produced fragments similar or identical in size to the fragments observed during activation of these molecules. None of seven normal individuals, and 29 of 99 patients with other forms of pulmonary disease contained measurable enzymes. Results showed that the proteolytic activity in BAL fluid of ARDS patients was blocked by diisopropylphosphofluoridate, Trasylol, soybean trypsin inhibitor, and normal plasma, or plasma deficient in inhibition of the first component of complement. Alpha$_1$-proteinase inhibitor (α1-PI)-deficient plasma failed to inhibit the proteolytic activity and addition of α1-PI to the deficient plasma reconstituted the inhibition. As seen here, much of the proteolytic activity of the BAL fluid from ARDS patients was identified as neutrophil elastase. In summary, the data presented here reveal that in BAL fluids from all 24 patients with ARDS, leukocytic elastase and/or α1-PI exist. A complex of elastase and α1-PI was observed in BAL fluids, and in some cases where active enzyme and α1-PI coexisted, free but inactive α1-PI was present.

McGuire, W. W., Spragg, R. G., Cohen, A. B., and *Cochrane, C. G.*

Journal of Clinical Investigation 69:543-553, 1982.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA; Pulmonary Division, University of California at San Diego, La Jolla, and the Pulmonary Division, Temple University Health Sciences Center, Philadelphia.

A PROTEASE-LIKE PERME
IMMUNOLOGIC IDENTITY

Extracts of guinea pig s
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Hageman factor in the skin ps
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space remains in question.

Yamamoto, T. and *Cochrane,*

American Journal of Patholog

Other support: National Institu

From the Department of Immu
Jolla, CA.

CELL-FREE TRANSLATION

In this attempt to cover f
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tem, is described.

A PROTEASE-LIKE PERMEABILITY FACTOR IN GUINEA PIG SKIN: IMMUNOLOGIC IDENTITY WITH PLASMA HAGEMAN FACTOR

Extracts of guinea pig skin have been shown previously to contain a potent permeability factor, while activated Hageman factor (HFa) is known to induce increased vascular permeability of considerable potency. With these things in mind, the experiment presented here was undertaken to determine specifically whether the permeability factor of skin extracts and Hageman factor are identical. Results of this study showed that vascular permeability enhancement activity of the protease-like permeability factor derived from guinea pig skin and of active guinea pig Hageman factor (αHFa) were both inhibited by anti-guinea pig Hageman factor rabbit $F(ab')_2$ antibody. The permeability activity of both factors was also absorbed on anti-Hageman factor $F(ab')_2$-Sepharose beads. The latent form of the permeability factor derived from skin extracts produced a single immunoprecipitation line with anti-Hageman factor and gave a reaction of identity with a precipitation band developing between purified Hageman factor and anti-Hageman factor. The latent permeability factor in the fraction corrected the clotting activity of Hageman-factor-deficient human plasma. The clotting activity was also blocked by anti-Hageman factor $F(ab')_2$ antibody. From these results, it was concluded that the skin permeability factor was immunologically and functionally indistinguishable from Hageman factor of plasma. In another study presented here, experiments using ^{125}I-Hageman factor showed that only 4 percent of the total Hageman factor in the skin pseudoglobulin fraction was derived from the vascular space in the harvested skin while the location of the Hageman factor in the interstitial space remains in question.

Yamamoto, T. and *Cochrane, C. G.*

American Journal of Pathology 107:127-134, 1982.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.

CELL-FREE TRANSLATION OF ELASTIN mRNAs

In this attempt to cover fully the system for effective cell-free translations of elastin mRNAs, the methodology is divided into four sections dealing with: (a) isolation of RNA and preparation of mRNA; (b) preparation of the mRNA-dependent reticulocyte lysate and optimal conditions for the cell-free translation of elastin mRNAs; (c) identification of tropoelastins in a cell-free translation assay; and (d) quantitation of tropoelastins in the cell-free translation system. Importantly, it is noted at this point that this discussion of the cell-free synthesis of elastin involves the synthesis of two proteins designated tropoelastins a and b. All the evidence to date suggests strongly that these two tropoelastins are separate gene products. Consequently, the methodologies described here are geared not only for the production of soluble elastin, but also for the separate identification of tropoelastins a and b. Sections of this paper are devoted to Isolation of Elastin mRNAs, Preparation of Lysate, Translation Conditions, Detection of Translated Tropoelastins, and Quantitation of Translated Tropoelastins, wherein rocket immunoelectrophoresis, a very sensitive technique for the direct quantitation of tropoelastins synthesized in the cell-free system, is described.

Foster, J. A. et al.

Methods in Enzymology 82:731-743, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

PREPARATION OF ANTISERUM TO TROPOELASTIN

The availability of antibody to soluble elastin is invaluable for detection, quantitation, and isolation of tropoelastin in a variety of *in vivo* and *in vitro* systems. The advantage to eliciting antiserum to tropoelastin is that the resulting antiserum contains precipitating antibody. The precipitin reaction between antibody and antigen is a prerequisite for performing various immunodiffusion and immunoelectrophoretic techniques available. In the preparation of antiserum, purified tropoelastin is injected intracutaneously into rabbits each week for a month. After a rest period of three weeks, another injection is given, and the rabbits are bled six-eight days later. The presence of precipitating antibody can be easily be tested by immunodiffusion. Specific antibody to tropoelastin can be prepared by affinity chromatography. The ideal ligand to attach onto a resin would be tropoelastin itself. However, because tropoelastin is very difficult to isolate in sufficient amounts, tryptic peptides released from lathyritic, insoluble elastin are an excellent alternative. In order to isolate tryptic peptides from the insoluble elastin, two different temperatures are used consecutively for trypsin digestion. In an attempt to bind specific tropoelastin antibody, 0.5 ml of chick tropoelastin antiserum is put onto a glass column and allowed to penetrate fully into a specifically prepared gel. The column is shut off for 15 minutes, then elution of serum proteins and nonelastin immunoglobulins are accomplished with 0.01 *M* Na_2HPO_4, 0.14 *M* NaCl(pH 7.4). Elution of the specific tropoelastin antibody is accomplished with 0.01 *M* sodium citrate, pH 2.8. It has been seen here that the antibody elutes immediately with this buffer and can easily be tested by immunodiffusion or immunoprecipitation.

Foster, J. A. et al.

Methods in Enzymology 82:762-765, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

ELASTIN GENE EXPRESSION

The scope of this article encompasses recent data relating to the molecular biology of elastin and the implications of these data to elastin biosynthesis. Since there are relatively few published reports in this area, a more general description of available techniques is presented, with a special emphasis on application to elastin. Included in this article are discussions of the isolation of elastin mRNAs, the optimal conditions for the cell-free translation of those mRNAs, procedures for the identification of elastin polypeptides directed by the synthesis of mRNA, the signal peptide found on the initially translated product, the differential expression of elastin mRNAs in various tissues during development, and the synthesis and cloning of elastin cDNA. Major sections of this paper deal with Isolation of Elastin mRNA; Cell-Free Translation of Elastin mRNAs; Identification of Tropoelastin; Tropoelastin Synthesis in Organ Cul-

ture; The Signal Peptide of Trop
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Foster, J. A., Rich, C. B. and

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Other support: National Institu

From the Department of Biolog

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suggests diverse contributions o
sensitivity reactions.

Phillips, M. J., *Gold, W. M.* an

The Journal of Immunology 131(

Other support: Medical Resea
Service and the National Institut

From the Howard Hughes Med
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Cell-Free Translation of
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ture; The Signal Peptide of Tropoelastin B; Differential Expression of Tropoelastin A and B during Development; Effect of Glucocorticoids on Elastin Synthesis, and Synthesis and Cloning of Elastin cDNA. In conclusion, the data discussed regarding the translation of elastin mRNA, the regulation of this translation during development, and the current research involving the cloning of elastin cDNA fragments represent a good beginning to an understanding of elastin gene expression and regulation.

Foster, J. A., Rich, C. B. and Karr, S. R.

International Review of Connective Tissue Research 10:65-95, 1983.

Other support: National Institutes of Health.

From the Department of Biology, Syracuse University, Syracuse, NY.

IgE-DEPENDENT AND IONOPHORE-INDUCED GENERATION OF LEUKOTRIENES BY DOG MASTOCYTOMA CELLS

Dog mastocytoma cells sensitized with dog anti-ragweed IgE and challenged with ragweed antigen or stimulated with calcium ionophore A23187 generate LTC_4, lesser quantities of LTD_4 and LTB_4, and several mono-HETEs of which 12-HETE and 15-HETE are the principal products. In this study, the maximum production of leukotrienes evoked by antigen challenge was attained within 5 min, and was maintained for up to 45 min. In contrast, the maximum production of leukotrienes by mastocytoma cells activated with optimum concentrations of 0.2 μM to 1.0 μM calcium ionophore was realized only after 30 min at 37°C. The maximum levels of LTC_4 achieved by stimulation with calcium inophore were approximately 50% higher than those attained by antigen challenge, whereas the difference for LTB_4 was approximately 35%. Irrespective of the stimulus, the quantity of LTC_4 was 3 to 4 times greater than that of LTD_4 and 2 to 2½ times greater than that of LTB_4. The characteristics of generation of mono-HETEs by mastocytoma cells activated with calcium ionophore and antigen challenge were similar. The level of 12-HETE attained a maximum after 10 min and the quantities of 15-HETE and 5-HETE reached the highest levels after 30 min with both stimuli. The leukotriene products of mastocytoma cells were identified by high-performance liquid chromatography, spectral properties, and immunoreactivity with mono-specific antisera, and exhibited the same concentration-dependence of biologic activity as authentic synthetic standards. The rapid maximum response to IgE-dependent stimulation and the unique spectrum of products distinguishes the secretion of leukotrienes by dog mastocytoma cells from that by basophils and some other types of mast cells, and suggests diverse contributions of mast cell leukotrienes to immediate and late hypersensitivity reactions.

Phillips, M. J., *Gold, W. M.* and Goetzl, E. J.

The Journal of Immunology 131(2):906-910, 1983.

Other support: Medical Research Council of Great Britain, U. S. Public Health Service and the National Institutes of Health.

From the Howard Hughes Medical Institute Laboratories, Cardiovascular Research Institute, and Department of Medicine, University of California Medical Center, San Francisco.

STIMULUS SPECIFICITY OF THE GENERATION OF LEUKOTRIENES BY DOG MASTOCYTOMA CELLS

For the work presented here, the availability of dog mastocytoma cells of high purity that release histamine and leukotrienes (LT) B_4, C_4, and D_4 in response to antigen and ionophore A23187 has permitted an initial comparative analysis of the specificity of activation of the two compartments of mediators by distinct stimuli. Results showed that isolated dog mastocytoma cells sensitized with dog anti-ragweed IgE and challenged with ragweed antigen or incubated with ionophore A23187 or the carboxy-terminal dodecapeptide of platelet factor 4, PF4(59-70), release histamine and concurrently generate leukotrienes B_4, C_4, and D_4. In contrast, the exposure of mastocytoma cells to 0.1-3μg/ml of 15-hydroxyeicosatetraenoic acid (15-HETE) stimulates selectively the generation of leukotrienes, in the absence of histamine release, while 0.1-1 μg/ml of compound 48/80 releases histamine without enhancing the generation of leukotrienes. The observation that natural stimuli are capable of selectively activating one synthetic or secretory compartment of mast cells suggests that separate subsets of receptors as well as different biochemical events may serve to mobilize each class of mediators.

Goetzl, E. J., Phillips, M. J. and *Gold, W. M.*

Journal of Experimental Medicine 158:731-732, 1983.

Other support: National Institutes of Health, Medical Research Council of Great Britain and the U. S. Public Health Service.

From the Howard Hughes Medical Institute Laboratories, Cardiovascular Research Institute, and Department of Medicine, University of California Medical Center, San Francisco.

SERIAL CHANGES IN MARKERS OF DISEASE ACTIVITY WITH CORTICOSTEROID TREATMENT IN SARCOIDOSIS

Sarcoidosis is a systemic granulomatous disease of unknown cause that may lead either to pulmonary fibrosis and respiratory impairment or to spontaneous resolution. The great variability of the clinical course has made it difficult to assess clinically the effectiveness of corticosteroid treatment, the mainstay of therapy, on the disease process. Consequently, a variety of independent parameters of disease activity such as serum angiotensin-converting enzyme levels, ^{67}gallium lung scanning, and bronchoalveolar lavage have been recommended as useful adjuncts in the clinical management of patients with sarcoidosis. The study presented here was designed to prospectively assess changes in clinical status, pulmonary function studies, and routine simple clinical tests, such as measurements of serum gamma globulin levels and erythrocyte sedimentation rate, as well as more complex or less widely available studies, including ^{67}gallium lung scanning, measurement of serum angiotensin-converting enzyme levels, and bronchoalveolar lavage fluid analysis in a group of 12 patients with active disease treated with corticosteroids. While all individual parameters of disease activity improved statistically with therapy, there were individual differences among the patients as to which test(s) was the more reliable indicator. In general, changes in ^{67}gallium lung scanning scores most closely reflected changes in clinical status, although measurements of serum angiotensin-converting enzyme levels were also very reliable. However, the number of bronchoalveolar lavage fluid IgG-secreting cells

remained elevated in the absence o
bronchoalveolar lavage fluid lymph
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fluid analysis, in sarcoidosis.

Lawrence, E. C. et al.

The American Journal of Medicin

Other support: The American Lun
Kelsey-Seybold Foundation, and
Service.

From the Rockwell-Keough Pulm
Clinical Center of The Methodist H
College of Medicine, Houston.

REGULATORY PEPTIDES AND
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Polak, J. M. and Bloom, S. R.

Experimental Lung Research 3(3&

From the Department of Histopath

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EURON SPECIFIC ENOLASE (
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remained elevated in the absence of clinically apparent disease, and the percentage of bronchoalveolar lavage fluid lymphocytes was often normal in patients who responded to corticosteriod therapy. Larger prospective studies are warranted to more extensively evaluate various measurements of disease activity, especially bronchoalveolar lavage fluid analysis, in sarcoidosis.

Lawrence, E. C. et al.

The American Journal of Medicine 74:747-756, 1983.

Other support: The American Lung Association, the National Institutes of Health, the Kelsey-Seybold Foundation, and the Veterans Administration Medical Research Service.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Center of The Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

REGULATORY PEPTIDES AND NEURON-SPECIFIC ENOLASE IN THE RESPIRATORY TRACT OF MAN AND OTHER MAMMALS

The early suggestion that a regulatory neuroendocrine system is present in the respiratory tract has now been shown to be a reality, confirmed by the finding of potently active peptides in both the autonomic nerves and mucosal APUD cells. Further verification is provided by the presence of a glycolytic enzyme, neuron-specific enolase (NSE), which can readily be immunostained to mark all components of this system in a single tissue section. The short review presented here deals with the distribution and cellular localization in the respiratory tract of five regulatory peptides (substance P, bombesin, vasoactive intestinal polypeptide (VIP), cholecystokinin, and somatostatin) and of the newly discovered neuroendocrine enzyme marker, NSE. Bombesin is found in typical mucosal endocrine cells, whereas the other regulatory peptides – principally substance P and VIP – are found, in significant concentrations, in autonomic nerves of the walls of the airways. Substance P, a putative sensory neurotransmitter, is found in autonomic nerves closely associated with the mucosal epithelium and the bronchial smooth muscle. VIP nerves, on the other hand, appear predominantly to innervate blood vessels, seromucous glands of the upper respiratory tract and bronchial smooth muscle. The presence of NSE in both mucosal APUD cells and autonomic nerves has established this neuronal enzyme as a useful marker for the entire neuroendocrine system of the lung and its derivative neoplasms.

Polak, J. M. and Bloom, S. R.

Experimental Lung Research 3(3&4):313-328, 1982.

From the Department of Histopathology, Hammersmith Hospital, London, England.

NEURON SPECIFIC ENOLASE (NSE) IMMUNOSTAINING A USEFUL TOOL FOR THE LIGHT MICROSCOPICAL DETECTION OF ENDOCRINE CELL HYPERPLASIA IN ADULT RATS EXPOSED TO ASBESTOS

As reported in this methodological paper, neuron specific enolase (NSE), an isoenzyme of the glycolytic enzyme enolase, has been immunostained in the endocrine

cells of the lung. Application of this method at light microscopical level clearly shows endocrine cell hyperplasia in the lungs of adult rats exposed to asbestos. Specifically, a marked endocrine cell hyperplasia at light microscopical level was revealed in the lungs of adult rats exposed to asbestos using antibodies to NSE. Very large groups of NSE-immunoreactive cells (20-80) were only observed in the lungs of rats exposed to asbestos for 12 months. In addition, smaller groups of cells (2-10) known to be present normally and to decrease with age, were rarely noted in the controls but were detected frequently in the treated rats. Therefore, immunoreactive NSE is a very good marker for endocrine cell hyperplasia and of early neoplastic changes.

Sheppard, M. N., Johnson, N. F., Cole, G. A., Bloom, S. R., Marangos, P. J., and *Polak, J. M.*

Histochemistry 74:505-513, 1982.

Other support: Wellcome Trust and the M.R.C. (UK).

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London; Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, Wales; and the Clinical Psychobiology Branch, National Institute of Mental Health, Bethesda, MD.

DISTRIBUTION OF REGULATORY PEPTIDES IN THE RESPIRATORY TRACT OF MAN AND MAMMALS

For the review presented here, the distribution of five regulatory peptides, vasoactive intestinal polypeptide (VIP), substance P, bombesin, cholecystokinin (CCK), and somatostatin, was investigated in man (adult and neonate) and in mammals (guinea pig, rat and cat) using immunocytochemistry and radioimmunoassay. Results showed that VIP and substance P, localized by immunocytochemistry to the lung innervation, were the most abundantly distributed. Bombesin, mainly localized in mucosal endocrine cells, was present in somewhat lower concentrations. The quantities of somatostatin and CCK were below the limits of immunocytochemical detection. The characteristic pattern of distribution of VIP and substance P in the lung innervation, and their proposed origin, is in keeping with their sets of actions. VIP is known to be a vasodilator, muscle relaxant and secretomotor neurotransmitter, whereas substance P, contained in nerve fibers that are shown in this study to originate from primary sensory neurons of the nodose ganglion, is regarded as a sensory neurotransmitter. In addition, some lung endocrine tumors were shown to contain significantly large concentrations of regulatory peptides, in particular bombesin, which was found to be present in numerous oat cell carcinomas. In conclusion, the use of antibodies to neuron-specific enolase is proposed, in this study, as the best tool for the visualization of the diffuse neuroendocrine system in its entirety, both in normal tissues and in disease states, particularly neuroendocrine neoplasias.

Polak, J. M. and Bloom, S. R.

In: Bloom, S. R., Polak, J. M. and Lindenlaub, E. (eds.): *Systemic Role of Regulatory Peptides*, New York: F. K. Schattauer Verlag, 1982, pp. 241-269.

Other support: The Wellcome Trust, UK.

From the Department of Histopathology, Hammersmith Hospital, London, England

IMMUNOCYTOCHEMICAL
ISLETS OF LANGERHANS

Previous radioimmunob
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Alejandro, R., Shienvold, F.

Diabetes 31(suppl. 4):17-22,

Other support: National Inst
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From the Departments of M
Medicine, Miami, FL.

TISSUE CULTURE REDU
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In this study it is shown
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IMMUNOCYTOCHEMICAL LOCALIZATION OF HLA-DR IN HUMAN ISLETS OF LANGERHANS

Previous radioimmunobinding assay studies have shown that human islet-enriched fractions possess HLS-A, -B, -C and DR antigens and that they are capable of eliciting allogeneic lymphoproliferative responses. The present study was undertaken to identify directly the cell type(s) that carries DR antigens in human islets, using ultrastructural immunocytochemistry with a nonpolymorphic monoclonal anti-DR antibody. In this study the presence of DR in the islet-enriched fractions (IEFs) was first demonstrated by radioimmunobinding assay. Light microscopic immunocytochemistry, in frozen sections of intact (unfixed) human pancreas, revealed a staining pattern suggestive of a vascular distribution of DR in islets. Ultrastructural localization of DR was then carried out by indirect immunoperoxidase labeling in the presence of NaN_3 (to prevent internalization of bound αDR). The major site of DR expression in the islet was the endothelial cell surface. Endocrine cells were devoid of αDR binding. Nonislet endothelium was also heavily labeled, but acinar and ductal cells were completely negative. Leukocytes bound αDR but were relatively rare in the IEFs. Human islets, therefore, clearly express HLA-DR, but predominantly on insular endothelial cells. Isolation of pure endocrine cell populations specifically free of endothelium would appear to be a rational approach to reducing immunogenicity in allogeneic transplantation.

Alejandro, R., Shienvold, F. L., Hajek, S. V., *Ryan, U.*, Miller, J., and Mintz, D. H.

Diabetes 31(suppl. 4):17-22, 1982.

Other support: National Institutes of Health, the Diabetes Research Institute Foundation, and Veterans Administration Research Support.

From the Departments of Medicine and Surgery, University of Miami School of Medicine, Miami, FL.

TISSUE CULTURE REDUCES Ia ANTIGEN-BEARING CELLS IN RAT ISLETS AND PROLONGS ISLET ALLOGRAFT SURVIVAL

In this study it is shown that lymphocytes, macrophages and capillary endothelial cells in rat islets of Langerhans bear Ia antigenic determinants, that these cells are reduced by culture, and that this is accompanied by a significant decrease of Ia antigen density as determined in a sensitive radioimmunobinding assay. To be precise, after it was shown here that rat pancreatic islet allograft survival was prolonged by a prior 7-day period of tissue culture, attention was drawn to identifying those cells in islets capable of stimulating allograft rejection (Ia antigen-bearing cells) and to determining whether such cells and/or their Ia antigens might be reduced by tissue culture. Freshly isolated and 7-day-cultured Wistar-Furth rat islets were incubated with a mouse anti-rat Ia nonpolymorphic monoclonal antibody, then with peroxidase-conjugated goat anti-mouse antibody, and processed for electron microscopy. Peroxidase (Ia)-positive lymphocytes, macrophages, and capillary endothelial cells were identified in fresh but not in cultured islets. A radioligand assay, using ^{125}I-protein A, revealed a 45% decrease in binding of Ia antibody to cultured compared with fresh islet cells. It was concluded, therefore, that Ia antigen-bearing lymphocytes, macrophages, and capil-

lary endothelial cells in rat islets are reduced by tissue culture and that this may account, at least in part, for the decreased immunogenicity of cultured islet allografts.

Rabinovitch, A., Alejandro, R., Noel, J., Brunschwig, J-P., and *Ryan, U. S.*

Diabetes 31(suppl. 4):48-54, 1982.

Other support: National Institutes of Health, NIAMDD Institutional Training Grant, and the Diabetes Research Institute Foundation.

From the Division of Endocrinology and Metabolism, the Department of Medicine, and the Department of Pharmacology, University of Miami School of Medicine, Miami, FL.

MATHEMATICAL ANALYSIS OF ENDOTHELIAL SIBLING PAIR CELL-CELL INTERACTIONS USING TIME-LAPSE CINEMATOGRAPHY DATA

In the study presented here, the division and migration behavior of endothelial cells under two differing culture conditions were analyzed by time-lapse cinematography. It was seen that in response to mechanical wounding of an intact monolayer, a relatively small proportion of the remaining cells immediately divide and migrate to fill the denuded area. However, the rates of division and migration of these cells are higher than for cells in a regular culture during growth to confluency. In addition in the wounded culture, sibling pairs tend to migrate in parallel and divide in synchrony. As repair of the wound proceeds, the migration behavior of cells in the wounded culture takes on characteristics of the regular culture, *e.g.* siblings migrating in opposite directions. As confluency is reached in both cultures cell division and migration cease. It was significant that in the wounded culture the first generation of siblings were very close (less than 150 μm apart) at division. Overall, the behavior differences between the two cultures resulted in a higher rate of increase in cell numbers, and thus faster repair, of the wounded monolayer.

Brown, L. M., *Ryan, U. S.*, Absher, M., and Olazabal, B. M.

Tissue & Cell 14(4):651-655, 1982.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL; Newnham College, Cambridge, England, and the Department of Medicine, University of Vermont School of Medicine, Burlington.

A SINGLE MONOCLONAL ANTI-Ia ANTIBODY INHIBITS ANTIGEN-SPECIFIC T CELL PROLIFERATION CONTROLLED BY DISTINCT Ir GENES MAPPING IN DIFFERENT H-2 I SUBREGIONS

Monoclonal antibodies (MAb), which are powerful reagents for both preparative and analytic purposes, have proven extremely useful in experiments aimed at elucidating the molecular basis of immune response (Ir) gene function and the mechanism of Ia-restricted T cell activation. In the work reported here, a xenogeneic rat anti-mouse Ia monoclonal antibody, M5/114 ($\gamma 2b,\kappa$), was studied for its effects *in vitro* on T cell

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Germain, R. N., Bhattachar

The Journal of Immunology

Other support: National Ins

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MAC-1 ANTIGEN: QUANT
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The Journal of Immunology

Other support: U. S. Public

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proliferative responses. Strain distribution studies revealed that M5/114 could inhibit I-A subregion-restricted T cell responses of the H-$2^{b,d,q,u}$ but not the N-$2^{f,s,v}$ haplotypes, indicating that this xenoantibody recognizes a polymorphic determinant on mouse Ia molecules. This same monoclonal antibody was found to inhibit BALB/c (H-2^{d}) T cell proliferation to both $G^{60}A^{30}T^{10}$ and $G^{58}L^{30}\phi^{4}$. The Ir genes regulating responses to these antigens map to either the I-A subregion (GAT), or the I-A and I-E subregions (GLø), raising the possibility that M5/114 recognizes both I-A and I-E subregion-encoded Ia glycoproteins. It could be shown, using appropriate F_1 responding cells, that M5/114 does in fact affect GAT and GLø responses by interaction with both the I-A and the I-E subregion products and not by any nonspecific effect resulting from binding to the I-A subregion product alone. These results are consistent with genetic and biochemical studies directly demonstrating that M5/114 recognizes $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecular complexes. The existence of a shared epitope on I-A and I-E subregion products suggests the possibility that these molecules arose by gene duplication. Finally, the precise correlation between the Ia molecules recognized by M5/114 and the ability of this antibody to block T cell responses under IR gene control strengthens the hypothesis that Ia antigens are Ir gene products.

Germain, R. N., Bhattacharya, A., Dorf, M. E., and *Springer, T. E.*

The Journal of Immunology 128(3):1409-1413, 1982.

Other support: National Institutes of Health.

From the Department of Pathology, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Havard Medical School, Boston.

MAC-1 ANTIGEN: QUANTITATIVE EXPRESSION IN MACROPHAGE POPULATIONS AND TISSUES, AND IMMUNOFLUORESCENT LOCALIZATION IN SPLEEN

As reported in this paper, the expression and structure of Mac-1 on peritoneal resident macrophages and on macrophages elicited by different agents have been examined by immunofluorescence, quantitative site number determination and immunoprecipitation. The expression of Mac-1 has been compared to that of Ia, which shows differential expression on macrophages depending on the eliciting agent. The tissue distribution of Mac-1 has also been examined by absorption, and immunofluorescence was used to localize Mac-1 bearing cells in spleen sections. The results show that Mac-1 is expressed on all types of macrophages examined, validating the use of Mac-1 as a general marker for distinguishing macrophages from lymphocytes and suggesting that it must have a generalized role in macrophage function. Mac-1 expression cannot be detected in lymph node cells or in the periarteriolar lymphoid sheath region of spleen but is found in the marginal zone and red pulp. The results also show that Mac-1 is synthesized by macrophages and is present on the cell surface in sufficient quantity for biochemical purification and characterization.

Ho, M-K. and *Springer, T. A.*

The Journal of Immunology 128(5):2281-2286, 1982.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

ANTI-MAC-1 SELECTIVELY INHIBITS THE MOUSE AND HUMAN TYPE THREE COMPLEMENT RECEPTOR

Monoclonal antibodies (MAb) have proven to be of great value in identifying the cellular lineages and subsets that give rise to the diversity of the immune system. Recently, interest has focused particularly on the use of such antibodies to evaluate macrophage heterogeneity. In the study reported here, anti-Mac-1 (M1/70), a rat monoclonal antibody that reacts with mouse and human macrophages, polymorphonuclear leukocytes (PMNL), and natural killer cells, selectively inhibited complement receptor-mediated rosetting by murine macrophages and human PMNL. Preincubation of macrophages with anti-Mac-1 inhibits formation of rosettes with sheep erythrocytes bearing IgM antibody and murine C3 fragments. No inhibition was observed when other monoclonal antibodies that react with macrophages (such as anti-Ly5, anti-H-2, or anti-pan-leukocyte) were tested at 10-fold higher concentrations. Anti-Mac-1 did not affect macrophage Fc receptor-mediated rosetting. Erythrocytes bearing homogeneous human C3 fragments C3b (EC3b) or C3bi (EC3Bi) were used to test the specificity of the murine macrophage and human PMNL complement receptor inhibited by anti-Mac-1. In both cases, anti-Mac-1 inhibited CR_3-mediated rosetting of EC3bi but not CR_1-dependent rosetting of EC3b. The results show that Mac-1 is either identical to CR_3 or closely associated with CR_3 function. This is one of the first cases in which a monoclonal antibody-defined differentiation antigen has been associated with a specific cell surface function.

Beller, D. I., *Springer, T. A.* and Schreiber, R. D.

Journal of Experimental Medicine 156:1000-1009, 1982.

Other support: National Institutes of Health, American Heart Association and the American Cancer Society.

From the Department of Pathology, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, and the Department of Molecular Immunology, Research Institute of Scripps Clinic, La Jolla, CA.

MONOCLONAL ANTIBODIES SPECIFIC FOR RAT IgG1, IgG2a, AND IgG2b SUBCLASSES, AND KAPPA CHAIN MONOTYPIC AND ALLOTYPIC DETERMINANTS: REAGENTS FOR USE WITH RAT MONOCLONAL ANTIBODIES

A set of monoclonal antibodies (MAb) defining rat heavy chain subclasses and kappa chain monotypic and allotypic determinants was characterized in this study. Specifically, mouse monoclonal antibodies to rat IgG were obtained by fusion of immune SJL mouse spleen cells to NSI myeloma cells. Seven monoclonal antibodies were labeled with ^{125}I and studied as to specificity and avidity by using a panel of rat monoclonal antibodies both as inhibitors and target antigens in soft well plate and indirect cell binding assays. All MAb were selected for high avidity of 4×10^7 to $\geq 2 \times 10^9\ M^{-1}$. Four MAb were subclass-specific, RG11/39, RG7/1, and RG7/11 were absolutely specific for the Fc′ region of IgG1, IgG2a, and IgG2b, respectively. RG9/6 showed specificity for the Fab′ region of IgG2a but cross reacted with lower avidity with IgG2c. Three MAb reacted with rat kappa chains. RG7/9 defined a monotypic (common) kappa chain determinant. RG11/15 and RG7/7 were specific for allelic

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Springer, T. A. et al.

Hybridoma 1(3):257-271, 1982

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kappa 1a and kappa 1b determinants, respectively. The monotypic and kappa 1a allotypic determinants are topographically separated. The antibodies can be used as screening reagents in indirect cell binding assays. They have sensitivity similar to affinity-purified rabbit anti-rat IgG and more defined specificity. They do not crossreact with mouse or human IgG, making them particularly suitable companion reagents for rat anti-mouse or anti-human MAb. One MAb, RG7/7, strongly crossreacts with Syrian hamster IgG.

Springer, T. A. et al.

Hybridoma 1(3):257-271, 1982.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Laboratory of Membrane Immunochemisty, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

EXPRESSION AND INDUCTION *IN VITRO* OF MACROPHAGE DIFFERENTIATION ANTIGENS ON MURINE CELL LINES

Differentiation is a process involving the coordinated control of many genes to produce different cell types. Some of these changes affect the cell surface. To follow up on this, macrophage antigens were studied on 12 macrophage cell lines and variant clones, as well as on 11 other lines representative of a variety of hematopoietic lineages, to determine the relation of surface antigen expression to cell differentiation, maturation, and function. Biosynthetic labeling followed by immunoprecipitation with monoclonal antibodies and gel electrophoresis showed that Mac-1 polypeptides of 95,000 and 170,000 M, and the Mac-2 polypeptide of 32,000 M, were found in lysates of mature macrophage lines but not in other lines, including myeloid or immature leukemias. The Mac-3 antigen was found in large amounts in all macrophage lines and to lesser degrees in some myeloid and B lymphoid lines. The M, of Mac-3 varied from 100,000 to 170,000, perhaps due to differential glycosylation. Analysis of Mac-1 and Mac-2 antigens by flow cytometry showed expression on all macrophages lines. Similarly, all three Mac antigens were detected in high amounts on macrophage lines by ^{125}I-labeled antibody binding. Mac-1 and Mac-2 were not routinely seen on other hematopoietic lines, but Mac-3 was expressed in variably low amounts on some lines of myeloid and B cell lineage. Mac-1 and Mac-3 but not Mac-2 could be induced in the M1 myeloblast line by corticosteroid, lipopolysaccharide, and several conditioned media containing myeloid colony-stimulating activity. Although anti-Mac-1 does not block the detection of Mac-3 antigen on induced M1 cells, the presence of anti-Mac-1 antibody during induction partially blocked the appearance of Mac-3 antigen. Thus, the surface structure associated with Mac-1 appears to be involved in differentiation of macrophages.

Ralph, P., Ho, M-K., Litcofsky, P. B., and *Springer, T. A.*

The Journal of Immunology 130(1):108-114, 1983.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Department of Developmental Hematopoiesis, Memorial Sloan-Kettering Cancer Center, Rye, NY, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

QUANTITATION OF HYBRIDOMA IMMUNOGLOBULINS AND SELECTION OF LIGHT-CHAIN LOSS VARIANTS

In immunoglobulin-synthesizing cells, the genes for immunoglobulin heavy and light chains are expressed by one chromosome each, and the expression of allelic genes on the homologous chromosomes are excluded. In hybridoma cells, the active genes for immunoglobulin synthesis from both the myeloma and spleen cell parents continue to be expressed. Thus, two different heavy chains and two different light chains may be made by a single hybridoma cell. When compared to conventional antisera, hybridoma antibodies offer many advantages including specificity, consistency, availability in large quantities, and the ability to use impure immunogens. Hybridoma antibodies also differ in two other respects from conventional antibodies. First, some hybridomas secrete myeloma as well as specific antibody chains. Methods are described in Section I for selecting variant clones from mouse-rat or mouse-mouse hybrids that secrete homogenous immunoglobulins. Second, in addition to measuring antibody activity, it is often desired to measure monoclonal antibody immunoglobulin concentration. The specific antibody component of monoclonal antibodies consists of a single heavy-chain subclass and light-chain isotype. Monoclonal antibodies therefore express only a portion of the antigenic determinants found in whole immunoglobulins. This has important implications for the measurement of monoclonal immunoglobulin concentration by immunoassay. Section II describes methods for measuring rat or mouse monoclonal immunoglobulins derived from rat-mouse, mouse-mouse, or rat-rat hybrids.

Springer, T. A.

Methods in Enzymology 92-147-160, 1983.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

MONOCLONAL ANTIBODIES AS TOOLS FOR THE STUDY OF MONONUCLEAR PHAGOCYTES

The analysis of complex biological systems has been given great impetus recently by the myeloma X immune spleen hybrid technique of Köhler and Milstein. If, for example, mouse macrophages are injected into the rat, the resultant multispecific response to a large array of different macrophage surface molecules may be resolved by cloning into a set of hybrid lines, each secreting a monoclonal antibody (MAb) recognizing a single antigenic determinant on a single cell surface molecule. Recently, a substantial number of antimacrophage MAb have been obtained that are already proving to be invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function and surface antigen structure. The properties of monoclonal antibodies defining murine and human macrophage differentiation antigens are summarized in two tables presented in this report. Most antibodies have been characterized for expression on different leukocytes and cell lines, but not on nonhematopoietic tissues or on mononuclear phagocytes other than macrophages. None of these monoclonals binds to lymphocytes except 2.4G2, directed to the Fc receptor II, which

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Springer, T. A.

In: Adams, D., Edelson, P. an
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Other support: U. S. Public H

From the Laboratory of Membr
Harvard Medical School, Bos

TISSUE DISTRIBUTION, ST
BIOSYNTHESIS OF MAC-3
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Ho, M-K. and *Springer, T. A.*

The Journal of Biological Che

Other support: U. S. Public H

From the Laboratory of Membr
Harvard Medical School, Bost

is expressed on B but not on T lymphocytes. The anti-Mac-1 MAb (M1/70) cross-reacts with both mouse and human macrophages. Mac-1 in the mouse and human and OKM1 and Mol in the human show highly similar expression on macrophages, granulocytes, and null cells, suggesting they may define homologous antigens. As is pointed out here, the ability to obtain large quantities of monospecific antibodies after growth *in vivo* or *in vitro* is a great advantage of hybridoma lines.

Springer, T. A.

In: Adams, D., Edelson, P. and Koren, H. (eds.): *Methods for Studying Mononuclear Phagocytes*, New York: Academic Press, Inc., 1981, pp. 305-313.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

TISSUE DISTRIBUTION, STRUCTURAL CHARACTERIZATION, AND BIOSYNTHESIS OF MAC-3, A MACROPHAGE SURFACE GLYCOPROTEIN EXHIBITING MOLECULAR WEIGHT HETEROGENEITY

The cell distribution and biochemical characterization of the antigen, Mac-3 are described in this paper. Mac-3 is a mouse macrophage differentiation antigen defined by a rat anti-mouse monoclonal antibody (MAb), M3/84. The structure, biosynthesis, quantitative surface expression, and distribution of Mac-3 have been studied by radiolabeling and isolation with MAb-Sepharose, saturation binding, absorption, and immunofluorescence flow cytometry. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Mac-3 migrates as a diffuse band with average M_r = 110,000. Labeling of intact cells with ^{125}I and accessibility to MAb show it is present at least in part on the cell surface. Saturation labeling with ^{125}I-MAb shows 4.2×10^6 cell surface sites on thioglycollate medium-elicited peritoneal macrophages. [^{35}S]Methionine and [^3H]glucosamine incorporation into Mac-3 by purified macrophages show it is a glycoprotein synthesized by these cells. Absorption shows Mac-3 is strongest in macrophages, present in lower quantities in lung, liver, bone marrow and spleen, and undetectable in thymus, lymph node, brain, and heart. Immunofluorescent flow cytometry shows surface expression on thioglycollate-elicited macrophages but not bone marrow, spleen, lymph node or thymus cell suspensions. Similar amounts of Mac-3 are immunoprecipitated from resident macrophages or macrophages elicited by sterile inflammatory agents, intracellular parasites, or immunomodulators, but the average M_r of Mac-3 varies from 92,000 to 110,000. Mac-3 is synthesized from precursor(s) of M_r = 74,000 and 79,000, identical in the different macrophages. Processing into the mature molecules occurs in 15 to 30 min.

Ho, M-K. and *Springer, T. A.*

The Journal of Biological Chemistry 258(1):636-654, 1983.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

DISTRIBUTION OF ACTIN IN SPREADING MACROPHAGES: A COMPARATIVE STUDY ON LIVING AND FIXED CELLS

The spreading of macrophages on a substrate apparently involves changes in the distribution of several contractile and cytoskeletal proteins as revealed by immunofluorescence and electron microscopy. In the paper presented here, the distribution of actin in proteose peptone-elicited murine peritoneal macrophages is examined with fluorescent analog cytochemistry (FAC), immunofluorescence, and electron microscopy (EM). Results show, in summary, that the optimal approach for elucidating the distribution of cytoskeletal and contractile proteins involved in motile processes is a combination of three techniques. Immunofluorescence and electron microscopy can yield a great deal of information concerning the structural components of the cytoskeleton, while FAC allows the researchers to follow dynamic changes of both the soluble and structural pools of cytoskeletal proteins in living, functional cells. Results from one technique must be interpreted with caution due to the potential artifacts and limitations of each technique. The concomitant use of FAC and immunofluorescence can minimize artifacts such as local differences in pathlengths and accessible volume, thereby permitting qualitative determinations of the local concentrations of proteins in different regions of the cell.

Amato, P. A., Unanue, E. R. and *Taylor, D. L.*

The Journal of Cell Biology 96:750-761, 1983.

Other support: National Institutes of Health.

From the Department of Cellular and Developmental Biology, Harvard University, the Biological Laboratories, Cambridge, MA, and the Department of Pathology, Harvard Medical School, Boston.

PROSTAGLANDINS MODULATE MACROPHAGE Ia EXPRESSION

Prostaglandins (molecules derived from arachidonic acid of membrane lipids) are important modulators of inflammation and of humoral and cellular responses. In order to evaluate a possible mechanism for the regulation of immune responses, the effects of prostaglandins on the expression of I-region-associated (Ia) glycoproteins by macrophages have been studied. The expression of these glycoproteins is essential for macrophages to function as antigen-presenting cells during the induction of immune responses. The synthesis and membrane expression of Ia, however, is not a constitutive property of the phagocyte but is under regulation and a positive regulation of this process is exhibited by activated T cells. In contrast, a negative regulation is conspicuously found in the neonate where a product from a young replicating macrophage inhibits the expression of Ia by the mature macrophages. It is shown here that prostaglandins of the E series (PGE) are potent inhibitors of the expression of Ia-antigens on macrophages and that thromboxane B_1 antagonizes the effect of PGE.

Snyder, D. S., Beller, D. I. and *Unanue, E. R.*

Nature 299(5879):163-165, 1982.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

CORTICOSTEROIDS INHIB
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Snyder, D. S. and *Unanue, E*

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Other support: National Canc

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Woda, B. A. and McFadden,

Experimental Cell Research 1

Other support: From the Depa
ical Center, Worcester.

CORTICOSTEROIDS INHIBIT MURINE MACROPHAGE Ia EXPRESSION AND INTERLEUKIN 1 PRODUCTION

Corticosteroids have a broad range of effects on the tissue distribution and/or function of immunocompetent cells involved in the induction of immune responses. The investigators have now examined their effects on macrophage expression of I region-associated (Ia) molecules and production of interleukin 1 (IL 1), which are proteins required for antigen presentation and stimulation of T cells—processes essential for inducing responses to all polypeptide antigens. The major findings are that corticosteroids inhibited macrophage Ia expression, production of IL 1, and in accordance with these effects, the presentation of antigen for T cell proliferation. Lymphokine-induced Ia expression was inhibited both at the cellular level *in vitro* and at the population level *in vivo* by therapeutic doses of these drugs. In summary, corticosteroids have profound effects on functions of the macrophage associated with antigen presentation to T cells. The drugs inhibited the expression of surface I-region-associated (Ia) antigens by peritoneal macrophages both *in vitro* and *in vivo*, reduced the production of IL 1, and inhibited antigen presentation for T cell proliferation by macrophages. The doses of hydrocortisone and prednisolone that inhibited by 50% Ia expression in cultured macrophages ranged around 2 to 5 $\times$ 10^{-8}M. These results could explain one mechanism by which corticosteroids suppress the induction of immune responses.

Snyder, D. S. and *Unanue, E. R.*

The Journal of Immunology 129(5):1803-1805, 1982.

Other support: National Cancer Institute.

From the Department of Pathology, Harvard Medical School, Boston.

LIGAND-INDUCED CAPPING OF SURFACE PROTEINS ON TRIFLUOPERAZINE-TREATED MACROPHAGES

Peritoneal exudate macrophages directly endocytose cross-linked membrane proteins such as Con A receptors and histocompatibility proteins. Under identical conditions, lymphocytes and transformed macrophages cap these proteins. In the experiments presented here, it could be seen that macrophages endocytosed their membrane proteins after cross-linking. After treatment with trifluoperazine, a calmodulin antagonist which inhibits endocytosis, a proportion of macrophages capped their membrane proteins. With some ligands there appeared to be a concentration threshold which was necessary to initiate capping.

Woda, B. A. and McFadden, M. L.

Experimental Cell Research 140:447-454, 1982.

Other support: From the Department of Pathology, University of Massachusetts Medical Center, Worcester.

LATERAL MOBILITY AND CAPPING OF RAT LYMPHOCYTE MEMBRANE PROTEINS

Lymphocyte membrane proteins undergo polar migration or capping after cross-linking by antibodies. There are two separable types of membrane proteins based on their capping characteristics. The prototype of one class is surface immunoglobulin (SIg) which caps after binding of a single cross-linking ligand. RT1 (rat histocompatibility proteins) and thy-1 are members of a second class of integral membrane proteins which cap only after the addition of a second ligand. In this study, fluorescence recovery after photobleaching experiments shows that there is heterogeneity in the diffusion characteristics of lymphocyte membrane proteins. SIg is relatively immobile when labeled by Fab' and is immobilized by $F(ab')_2$. RT1 diffuses 2.7 times as fast as SIg and the mobile fraction of RT1 is greater. When RT1 is cross-linked by relatively low concentrations of $F(ab')_2$ its mobility is about the same as that of SIg. When the concentration of $F(ab)_2$ is increased, RT1 is immobilized. Thy-1 diffuses faster than SIg and has a higher mobile fraction. When thy-1 is labeled with $F(ab')_2$ it remains mobile and is immobilized when a second antibody layer is added. The data presented here show that while there appear to be only two classes of lymphocyte membrane protein capping, the lateral mobility of membrane proteins is more heterogeneous. The relative order of lateral mobility of membrane proteins is thy-1 >>RT1 >SIg.

Woda, B. A. and Gilman, S. C.

Cell Biology International Reports 7(3):203-209, 1983.

Other support: National Institutes of Health.

From the Department of Pathology, University of Massachusetts Medical Center, Worcester, and the Research Institute of Scripps Clinic, La Jolla, CA.

VII. *Epidemiology*

INFLUENCE OF CIGARETTE, PIPE, AND CIGAR SMOKING, REMOVABLE PARTIAL DENTURES, AND AGE ON ORAL LEUKOPLAKIA

In this epidemiological study, 925 healthy male subjects from the Veterans Administration Dental Longitudinal Study were examined for oral leukoplakia lesion site and prevalence. The participants were grouped according to smoking status: nonsmokers, smokers of cigarettes, cigars, pipe, or cigarettes plus another tobacco product. In addition, the amount of product smoked, presence of a removable partial denture, and age were examined to determine their relation to leukoplakia. Results showed that leukoplakia lesions existed in 127 (13.7%) of the total 925 persons. The 443 nonsmokers had a prevalence of 3.8%; the 482 total smokers had a lesion prevalence of 22.8%. Furthermore, the data indicated that cigar smokers had significantly fewer lesions than the other smoker groups and that persons smoking a pipe or a pipe plus cigars had the highest prevalence. Heavy cigarette smokers had significantly more palatal lesions than light to moderate cigarette smokers. While removable partial dentures did not

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Baric, J. M. *et al.* (*Bossé, R*

Oral Surgery 54(4):424-429,

Other support: Medical Res

From the Veterans Administ
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Cederlöf, R. *et al.* (*Rantasal*

International Journal of Epia

Other support: From the Dep
and the Department of Envir
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CIGARETTE SMOKING AN
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tly more palatal lesions
partial dentures did not

appear to affect lesion prevalence significantly, older smokers, persons of 50 years and above, had a significantly higher prevalence than persons less than 50 years of age.

Baric, J. M. *et al.* (*Bossé, R.*)

Oral Surgery 54(4):424-429, 1982.

Other support: Medical Research Service of the Veterans Administration.

From the Veterans Administration Outpatient Clinic and Harvard School of Dental Medicine, Boston.

A CROSS-NATIONAL EPIDEMIOLOGICAL RESOURCE: THE SWEDISH AND FINNISH COHORT STUDIES OF LIKE-SEXED TWINS

In 1961 a nationwide registry of 12,889 like-sexed twin pairs born between 1886 and 1925 was set up in Stockholm, Sweden; in 1973 this registry was extended to include like-sexed twin pairs born between 1926 and 1958. In 1974, a study of 17,357 like-sexed twin pairs born before 1958 and alive in 1967 was started in Helsinki, Finland. Both studies comprise an unselected series that has been studied in a comparable fashion. Zygosity determination and health questionnaire data-gathering were carried out in 1973 for the Swedish study and in 1975 for the Finnish study for the comparable age groups. Data on hospital usage, cancer incidence and mortality are collected by record-linkage from the respective national registries. Cross-national twin studies can permit testing of hypotheses of the relationships between genetic and cultural factors and major chronic diseases and their risk factors. Specifically, an immediate objective of this study is to present descriptive results relating to disease symptoms and behavioral characteristics for Sweden and Finland. Then the data collected can be used to estimate the importance of different environmental risk factors and genotype in explaining differences in morbidity and mortality between the two countries.

Cederlöf, R. *et al.* (*Rantasalo, I.* and *Floderus-Myrrhed, B.*)

International Journal of Epidemiology 11(4):387-390, 1982.

Other support: From the Department of Public Health Science, University of Helsinki, and the Department of Environmental Hygiene of the Karolinska Institutet and the National Institute of Environmental Medicine, Stockholm.

CIGARETTE SMOKING AND ALCOHOL USE IN FINLAND AND SWEDEN: A CROSS-NATIONAL TWIN STUDY

Finland and Sweden both have twin cohort studies. In this cross-national twin study, cigarette smoking and alcohol use in Finland and Sweden were compared using data from the two studies on like-sexed adult twin pairs aged 18-47 (total of 20,056 pairs). Results showed that Finnish men were heavier consumers of tobacco and alcohol than Swedish men. When heavy consumers (>500g of alcohol/month and >20 cigarettes/day) were considered, the prevalence rate was 9.7% in Finnish men and 5.1% in Swedish men. Since there is a higher morbidity in Finland than in Sweden

from many smoking- and alcohol-associated diseases, this difference might account for it. Genetic factors in smoking and alcohol use were assessed by comparing observed and expected coincidence rates, and by multivariate analyses. Genetic and familial effects were defined as an excess coincidence in monozygotic pairs compared to dizygotic (DZ) pairs, and by an increased DZ coincidence rate compared to that expected. Significant genetic and familial effects were observed for cigarette smoking and for smoking more than one pack of cigarettes a day. Significant familial effects for alcohol use were observed and a significant genetic effect was obtained for men. However, a significant genetic effect could not be observed for the combined heavy use of alcohol and heavy smoking. The genetic and familial effects seemed to be mostly independent of country and sex.

Kaprio, J. *et al.* (*Rantasalo, I.* and *Floderus-Myrrhed, B.*)

International Journal of Epidemiology 11(4):378-386, 1982.

Other support: From the Department of Public Health Science, University of Helsinki, and the Department of Environmental Hygiene of the Karolinska Institutet and the National Institutet of Environmental Medicine, Stockholm.

A SEMANTIC DIFFERENTIAL OF PSYCHOSOCIAL BEHAVIOUR PATTERNS

A behavioral self-assessment rating scale developed for use in questionnaire studies can also be used to assess characteristics of other persons well-known to the self (siblings, spouse, cotwin, etc.). The method used to construct the 43-item scale was the semantic differential technique. After a pilot sutdy was run using this scale, a slightly revised version was administered to six groups of working-aged persons in the Helsinki area (n = 238). The subjects' age, sex, occupation, educational level as well as education and occupation of their parents were recorded. A varimax-rotated factor analysis indicated that four factors could be identified with characteristic values >1.0. These factors, named according to the positive end of the reading, were: (1) spontaneity-openness, (2) self-control-calmness, (3) self-confidence-dominance, and (4) conscientiousness-responsibility. Analysis of covariance of the factor scores with background variables as independent variables showed that sex was the only significant explanatory variable for the first, second and fourth factors. A shortened version of the scale was constructed using the five items with the highest loadings on each factor. Item analyses yielded reliability estimates of 0.81, 0.77, 0.82, and 0.68 for the four shortened factor scales. Thus, a reliable behavioral self-assessment scale with four dimensions was developed that is based on using everyday words in a semantic differential. The factor structures seem to be independent of educational and occupational factors, but sex differences for three of the four scales were observed. This instrument, which has been used in the 1979 and 1981 questionnaire studies of the Finnish Twin Cohort Study, will continue to be used as a behavioral assessment tool in clinical and epidemiological studies.

Langinvainio, H. *et al.* (*Rantasalo, I.*)

Kansanterveystieteen julkaisuja M 68:1-43, 1982.

Other support: From the Department of Public Health Science, University of Helsinki.

HEREDITARY AND EI SLEEP LENGTH

An earlier survey c difference in sleep lengtl though it is known that limited, it is difficult to biological factors, while virtually unknown. In th length, the authors have c and dizygotic (DZ) twin consists of all adult like-s members alive and answ mined by questionnaire v 4,545 DZ. 1,328 of the p night sleep was 7 h 51 mi sexes and different age females averaged 11 min n DZ twin pairs living tog showing cohabitation to influenced by age and sex the latter factors were ass parameter. For short slee zygosity, cohabitation an long sleep (≥ 9 h) signific Self-reports on sleep lengt a constellation of undefine Thus, for example, in me to sleep quality and incom influenced by age and env genetic and possibly othe

Partinen, M. *et al.* (*Rant*

In: Proceedings of the 6th Basel: Karger, 1983, pp.

Other support: Finnish C

From the Departments of sity of Helsinki.

HEREDITARY AND ENVIRONMENTAL DETERMINATION OF HUMAN SLEEP LENGTH

An earlier survey on self-reported sleep characteristics has shown a definite difference in sleep length between Finnish and corresponding American youths. Although it is known that normative data on human sleep length are geographically limited, it is difficult to assess to what extent such differences reflect cultural and biological factors, while the genetic determinants of normal sleep in humans are virtually unknown. In this attempt to estimate the role of genetic factors on sleep length, the authors have compared the self-reported sleep length in monozygotic (MZ) and dizygotic (DZ) twins of the Finnish twin cohort study. The material used here consists of all adult like-sexed Finnish twin pairs (n = 11,368) born before 1958, both members alive and answering a questionnaire mailed in 1975. Zygosity was determined by questionnaire validated by blood sampling. 2,237 of the pairs were MZ and 4,545 DZ. 1,328 of the pairs lived together. Results showed that the overall mean of night sleep was 7 h 51 min. There were small but significant differences between the sexes and different age groups. Sex difference was greatest at 20-34 years when females averaged 11 min more sleep than males. Intraclass correlations for both MZ and DZ twin pairs living together were expectedly higher than for those living apart, showing cohabitation to be an important synchronizing factor. As cohabitation was influenced by age and sex, which also affected sleep, the relative roles of genetic and the latter factors were assessed by multivariate logit analysis for concordance of each parameter. For short sleep ($\geq$ 6 h) the logit analysis indicated significant effects for zygosity, cohabitation and age, with a significant interaction between age and sex. For long sleep ($\geq$ 9 h) significant effects were detected for zygosity, cohabitation and sex. Self-reports on sleep length show individual differences, which have been attributed to a constellation of undefined biophysiological, psychosocial and environmental factors. Thus, for example, in men of the present study population sleep length was correlated to sleep quality and income. These results indicate that whereas human sleep is greatly influenced by age and environment, one-third of the variance in sleep length is due to genetic and possibly other factors that make MZ twins more similar than DZ twins.

Partinen, M. *et al.* (*Rantasalo, I.*)

In: Proceedings of the 6th European Congress on Sleep Research, Zurich, 1982: *Sleep*, Basel: Karger, 1983, pp. 206-208.

Other support: Finnish Cultural Foundation.

From the Departments of Neurology, Physiology and Public Health Science, University of Helsinki.

Active Projects

Following is a list of the principal investigators, or institutions, of projects under way or activated in the period since the previous Report, together with the respective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
LEO G. ABOOD, PH.D., *Professor of Brain Research and Biochemistry*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Nicotine transfer-disposition in liver cells
KENNETH B. ADLER, PH.D., *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	Airway mucin secretion: effects of products from bacteria associated with chronic bronchitis
JOHN J. ALBERS, PH.D., *Research Associate Professor of Medicine*, University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D., *Professor of Biochemistry*, Harvard University School of Public Health, Boston.	Human platelet-derived growth factor (PDGF): relationship to human atherosclerosis
THOMAS M. AUNE, PH.D., *Adjunct in Immunology*, The Jewish Hospital of St. Louis.	Interferon—activation of suppressor T cell pathways
BERNARD M. BABIOR, M.D., PH.D., *Professor of Medicine*, New England Medical Center Hospital, Boston.	Studies on the mechanism of activation of the respiratory burst in neutrophils
LESLIE BAER, M.D., *Associate Professor of Medicine*, Columbia University College of Physicians & Surgeons, New York.	Cigarette smoking in normotensive and hypertensive subjects: blood pressure, renin, aldosterone and catecholamine responses
SAMUEL BALK, M.D., PH.D., *Pathologist*, New England Deaconess Hospital, Boston.	Serum mitogens, hormones, ions, viral transforming genes and tumor reversal in appropriate and autonomous initiation of cell replication
RICHARD J. BING, M.D., *Professor of Medicine (emeritus)*, University of Southern California School of Medicine, Los Angeles; *Visiting Associate*, California Institute of Technology; *Director of Experimental Cardiology and Scientific Development*, Huntington Medical Research Institute, Pasadena, CA.	Lipoproteins and the arterial wall
DEBAJIT K. BISWAS, PH.D., D.Sc., *Associate Professor of Oral Biology*, Laboratory of Pharmacology, Harvard School of Dental Medicine, Boston.	Effects of nicotine and benzo(α)pyrene on hormone production
IRA B. BLACK, M.D., *Professor and Chief, Division of Developmental Neurology*, Cornell University Medical College, New York.	Nicotine and neuronal development
PHYLLIS B. BLAIR, PH.D., *Professor of Immunology*, University of California, Berkeley.	Regulation of natural killer cell activity

PRINCIPAL INVESTIGATOR OR INSTITUTION
EDWARD BRESNICK, PH.D., *and Chairman, Department of try*, The University of Vermont Medicine, Burlington.
VINCENZO BUONASSISI, M.D *Research Biologist*, The Univer fornia at San Diego, La Jolla.
EDWARD J. CAMPBELL, M.D *Professor of Medicine*, Washing sity School of Medicine, St. Lo
FRANCIS C. CHAO, M.D., PH *Investigator*, Center for Blood Boston.
LAN BO CHEN, PH.D., *Associat of Pathology*, Sidney Farber C tute, Boston.
JAN F. CHLEBOWSKI, PH.D. *Professor of Biochemistry*, Medi of Virginia, Richmond.
CURT I. CIVIN, M.D., *Assistant I Oncology & Pediatrics*, The Joh Oncology Center, Baltimore.
BRIAN L. CLEVINGER, PH.D. *Professor of Biomedical Science* ton University School of Dental St. Louis.
CHARLES G. COCHRANE, M *ber, Department of Immuno* Scripps Clinic and Research F La Jolla, CA.
BERNICE H. COHEN, PH.D., *Pr Director, Human Genetics/Gen miology Program*, The Johns Ho versity School of Hygiene a Health, Baltimore.
ROBERT W. COLMAN, M.D., *P Medicine*, Temple University Medicine, Philadelphia.
CARL E. CRUETZ, PH.D., *Assist sor of Pharmacology*, Universi ginia School of Medicine, Charl
GIDON CZAPSKI, M.Sc., PH.D. *of Physical Chemistry*, The Hebr sity, Jerusalem, Israel.
ALBERT B. DEISSEROTH, M.I *Professor of Medicine*, Veterans tration Medical Center, San Fra
PETER H. DUESBERG, PH.D., *Molecular Biology*, University nia, Berkeley.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
EDWARD BRESNICK, PH.D., *Professor and Chairman, Department of Biochemistry*, The University of Vermont College of Medicine, Burlington.	Expression of cytochrome P450c
VINCENZO BUONASSISI, M.D., *Associate Research Biologist*, The University of California at San Diego, La Jolla.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
EDWARD J. CAMPBELL, M.D., *Assistant Professor of Medicine*, Washington University School of Medicine, St. Louis.	Modulators of inflammatory cell proteolytic activity
FRANCIS C. CHAO, M.D., PH.D., *Senior Investigator*, Center for Blood Research, Boston.	Platelet activation and blood hypercoagulability
LAN BO CHEN, PH.D., *Associate Professor of Pathology*, Sidney Farber Cancer Institute, Boston.	Studies on human oat cell carcinomas
JAN F. CHLEBOWSKI, PH.D., *Assistant Professor of Biochemistry*, Medical College of Virginia, Richmond.	Calorimetric investigation of proteinase-α_2 macroglobulin interaction
CURT I. CIVIN, M.D., *Assistant Professor of Oncology & Pediatrics*, The Johns Hopkins Oncology Center, Baltimore.	Biochemistry and function of human granulopoietic antigens
BRIAN L. CLEVINGER, PH.D., *Assistant Professor of Biomedical Science*, Washington University School of Dental Medicine, St. Louis.	Role of J segment in V segment expression
CHARLES G. COCHRANE, M.D., *Member, Department of Immunopathology*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease
BERNICE H. COHEN, PH.D., *Professor and Director, Human Genetics/Genetic Epidemiology Program*, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.	Genetic-epidemiologic characteristics of smokers and nonsmokers
ROBERT W. COLMAN, M.D., *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Initiation of plasma coagulation and kinin forming systems in man
CARL E. CRUETZ, PH.D., *Assistant Professor of Pharmacology*, University of Virginia School of Medicine, Charlottesville.	Role of protein phosphorylation in nicotine reduced catecholamine release
GIDON CZAPSKI, M.Sc., PH.D., *Professor of Physical Chemistry*, The Hebrew University, Jerusalem, Israel.	On the toxicity of oxygen and superoxide ion: is superoxide toxic?
ALBERT B. DEISSEROTH, M.D., PH.D., *Professor of Medicine*, Veterans' Administration Medical Center, San Francisco.	Study of altered alpha globin genes in leukemia and solid tumors
PETER H. DUESBERG, PH.D., *Professor of Molecular Biology*, University of California, Berkeley.	Transforming genes of two acute leukemia viruses

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
HAROLD F. DVORAK, M.D., *Chief, Department of Pathology*, Beth Israel Hospital, Boston.	Pathogenesis of tumor desmoplasia
V. GENE ERWIN, PH.D., *Professor of Pharmacology; Dean*, University of Colorado School of Pharmacy, Boulder.	Effects of nicotine on neuropeptide secretion by intact mouse brain, a pharmacogenetic study
ALVAN R. FEINSTEIN, M.D., *Professor of Medicine & Epidemiology*, Yale University School of Medicine, New Haven, CT.	Smoking, detection bias and primary lung cancer
RICHARD FENTON, PH.D., *Instructor in Physiology*, University of Massachusetts School of Medicine, Worcester.	Physiological effects of nicotine on calcium and adenosine metabolism by the heart
THOMAS H. FINLAY, PH.D., *Associate Professor of Obstetrics and Gynecology*, New York University Medical Center, New York.	Structure, properties and regulation of mouse plasma protease inhibitors
PAUL B. FISHER, PH.D., *Senior Research Associate, Department of Microbiology*, Columbia University College of Physicians & Surgeons, New York.	Chemical-viral interactions in cell transformation
JOSEPH A. FONTANA, M.D., PH.D., *Assistant Professor of Medicine and Biochemistry*, West Virginia University Medical Center, Morgantown.	Glycosyltransferases and glycoprotein synthesis in differentiation induced phenotypic reversal of malignancy by retinoic acid cyclic nucleotides and other agents
JUDITH ANN FOSTER, PH.D., *Professor and Chairperson, Department of Biology*, Syracuse University, Syracuse, NY.	Involvement of elastin in lung disease
JACK W. FRANKEL, PH.D., *Consultant in Medical Research*, Veterans Administration Medical Center, Bay Pines, FL.	Smoking and lung cancer: diagnostic test to identify persons at high risk
ALLAN P. FREEDMAN, M.D., *Assistant Professor of Medicine*, Hahnemann Medical College, Philadelphia.	The effect of cigarette smoking on the alveolar clearance rate of inert dust particles in the human lung
AARON E. FREEMAN, PH.D., *Staff Scientist*, California Biomedical Research Foundation, La Jolla, CA.	Rodent and human lung epithelial cell culture as a tool for carcinogenesis research *in vitro*
KJELL FUXE, M.D., *Professor of Histology*, The Karolinska Institute, Stockholm.	Nicotine, catecholamines and neuroendocrine functions
JACQUES E. GIELEN, PH.D., *Associate Professor, Laboratory of Medical Chemistry, Toxicology and Hygiene*, Institute of Pathology, University of Liège, Liège, Belgium.	Modulation of aryl hydrocarbon hydroxylase and epoxide hydratase in animal tissues and in cell culture by cigarette smoke condensate and other chemicals
	Biochemical mechanism(s) and qualitative and quantitative consequences of benzo-(α)pyrene metabolism
GABRIEL C. GODMAN, M.D., *Professor of Pathology*, Columbia University College of Physicians & Surgeons, New York.	Cytoskeletal organization of the endothelial cell in regulation of shape contractility and surface movement

PRINCIPAL INVESTIGATOR OR INSTITUTION
WARREN M. GOLD, M.D., *Medicine*, Cardiovascular Res tute, University of California cisco.
SIDNEY GOLDFISCHER, M.D *of Pathology*, Albert Einstein Medicine, The Bronx, NY.
MAURICE GREEN, M.D., *Di tute for Molecular Virology*, S versity Medical Center, St. Lo
CHARLES S. GREENBERG, M *ant Professor of Medicine*, Dul Medical Center, Durham, NC
MARK I. GREENE, PH.D., *A fessor of Pathology*, Harv School, Boston.
NOBUYOSHI HAGINO, M.D. *fessor of Anatomy*, Univers Health Science Center, San A
CAROLINE B. HALL, M.D., *A fessor of Pediatrics and Medi* sity of Rochester School Rochester, NY.
LINDA M. HALL, PH.D., *Asso sor of Genetics and Neurosc* Einstein College of Medicin University, The Bronx, NY.
PAUL HAMOSH, M.D., *Assoc of Physiology and Biophysic cine*, Georgetown Universit Medicine and Dentistry, Wasl
NORMAN W. HEIMSTRA, P *sor of Psychology; Director, tors Laboratory*, University kota, Vermillion.
ROBERT M. HOFFMAN, PH *Professor of Pediatrics in Re* versity of California School La Jolla.
JEROME L. HOJNACKI, PH *Professor of Biological Scie* sity of Lowell, Lowell, MA.
WAYNE HOSS, PH.D., *Associ* Center for Brain Research, Rochester Medical Center, R
HAROLD P. JONES, PH.D., *fessor of Biochemistry*, Univ Alabama, Mobile.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
WARREN M. GOLD, M.D., *Professor of Medicine*, Cardiovascular Research Institute, University of California, San Francisco.	Effect of ozone on airway mast cells
SIDNEY GOLDFISCHER, M.D., *Professor of Pathology*, Albert Einstein College of Medicine, The Bronx, NY.	Extracellular matrix-cytochemistry and ultrastructure
MAURICE GREEN, M.D., *Director, Institute for Molecular Virology*, St. Louis University Medical Center, St. Louis.	Amplification of human adenovirus transformation proteins in prokaryotic and eukaryotic cells
CHARLES S. GREENBERG, M.D., *Assistant Professor of Medicine*, Duke University Medical Center, Durham, NC.	Transglutaminases and atherosclerosis
MARK I. GREENE, PH.D., *Associate Professor of Pathology*, Harvard Medical School, Boston.	Suppressor cells in syngeneic tumor immunity
NOBUYOSHI HAGINO, M.D., PH.D., *Professor of Anatomy*, University of Texas Health Science Center, San Antonio.	Nicotinic receptors of LHRH axon terminals in the median eminence
CAROLINE B. HALL, M.D., *Associate Professor of Pediatrics and Medicine*, University of Rochester School of Medicine, Rochester, NY.	Interrelationship of infectious lower respiratory tract disease in infancy, and host and environmental factors to later development of chronic lung disease
LINDA M. HALL, PH.D., *Associate Professor of Genetics and Neuroscience*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Genetic differences in nicotine sensitivity in *Drosophila melanogaster* strains
PAUL HAMOSH, M.D., *Associate Professor of Physiology and Biophysics, and Medicine*, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.	Cigarette smoke and lipoprotein remodeling by the lung
NORMAN W. HEIMSTRA, PH.D., *Professor of Psychology; Director, Human Factors Laboratory*, University of South Dakota, Vermillion.	Some behavioral aspects of smoking and smoking deprivation
ROBERT M. HOFFMAN, PH.D., *Assistant Professor of Pediatrics in Residence*, University of California School of Medicine, La Jolla.	Methionine dependence, methylation and organic transformation Regulation of cellular oncogenes
JEROME L. HOJNACKI, PH.D., *Assistant Professor of Biological Sciences*, University of Lowell, Lowell, MA.	Nicotine-induced changes in primate high density lipoproteins
WAYNE HOSS, PH.D., *Associate Professor*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Studies of nicotine interaction with blood cells
HAROLD P. JONES, PH.D., *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Calcium-dependent regulatory proteins and neutrophil activation

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
MORRIS J. KARNOVSKY, M.B., B. Ch., *Shattuck Professor of Pathological Anatomy*, Harvard Medical School, Boston.	The molecular basis of pulmonary surfactant secretion by type II pneumocytes: studies in intact cells and a cell-free system
SIMON KARPATKIN, M.D., *Professor of Medicine*, New York University Medical Center, New York.	The role of platelets in tumor cell metastases
SHIRLEY L. KAUFFMAN, M.D., *Professor of Pathology*, State University of New York Downstate Medical Center, Brooklyn.	Oncogenes in chemical carcinogenesis
INGEGERD M. KEITH, PH.D., *Assistant Professor of Anatomy*, University of Wisconsin School of Veterinary Medicine, Madison.	Part I: Lung neuroendocrine cell innervation Part II: Transplacental effect of smoking on lung neuroendocrine cells in the neonate
HEINZ KOHLER, M.D., PH.D., *Director, Department of Molecular Immunology*, Roswell Park Memorial Institute, Buffalo, NY.	Multi-targeting with hybridomas on tumor cells
MARKKU KOSKENVUO, M.D., *Assistant Professor, Department of Public Health Science*, University of Helsinki, Helsinki, Finland.	The Finnish Twin Cohort Follow-up Study
ROBERT W. KREILICK, PH.D., *Professor of Chemistry*, University of Rochester, Rochester, NY.	Investigations of the interaction of nicotine with membranes
KLAUS E. KUETTNER, PH.D., *Professor and Chairman: Department of Biochemistry*, Rush College of Health Sciences and Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago.	Regulation of proliferation of invasive cells
ABEL LAJTHA, PH.D., *Director*, New York State Research Institute for Neurochemistry and Drug Addiction, New York.	Genetic basis for nicotine response
DON LAPENAS, M.D., *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	The association of inorganic dust deposition with pulmonary neoplasia in tobacco users
E. CLINTON LAWRENCE, M.D., *Assistant Professor of Medicine*, Baylor College of Medicine, Houston.	Effects of cigarette smoking on immunoglobulin production by human bronchial lymphocytes
PHILIP M. LE QUESNE, PH.D., D.SC., *Professor of Chemistry*, Northeastern University, Boston.	Assay of oxygenated sterols in human blood vessels – a feasibility study
GESINA L. LONGNECKER, PH.D., *Associate Professor of Pharmacology*, University of South Alabama College of Medicine, Mobile.	Studies of platelet and endothelial prostanoid production as possible cardiovascular risk indicators in smokers
RONALD B. LUFTIG, PH.D., *Professor and Head, Department of Microbiology and Immunology*, LSU Medical Center, New Orleans.	Interactions between RNA viruses and chemical carcinogens

PRINCIPAL INVESTIGATOR OR INSTITUTION
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RICHARD A. MARKHAM, M *Professor of Medicine and of and Immunology*, The Jewis St. Louis, St. Louis.
ALAN C. McLAUGHLIN, PH *in Biochemistry/Biophysics*, Pennsylvania School of Me delphia.
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CHRISTOPHER MURLAS, M *Professor of Medicine*, Univ fornia School of Medicine, I
JAY A. NADEL, M.D., *Prof cine, Physiology and Radi* vascular Research Institute, California, San Francisco.
DONALD J. NELSON, PH. *Professor of Chemistry*, Cl Worcester, MA.
STAFAN NIEWIAROWSKI, *Professor of Physiology*, T search Center, Temple Unive Medicine, Philadelphia.
F. WILLIAM ORR, M.D., As *sor of Pathology*, Universit Winnipeg, Manitoba, Canad
BENDICHT U. PAULI, D.V *Professor of Pathology*, Rus St. Lukes Medical Center,
BORIS M. PETERLIN, M.D. *fessor of Medicine*, Sectio tology-Clinical Immunology California School of Medic cisco.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
JAN M. LUNDBERG, M.D., *Assistant Professor of Pharmacology*, Karolinska Institutet, Stockholm, Sweden.	Sensory neuropeptides and smoke-induced irritation in the respiratory tract
HENRY T. LYNCH, M.D., *Professor and Chairman, Department of Preventive Medicine and Public Health*, Creighton University School of Medicine, Omaha.	Genetic and biomarker studies of cancers of the respiratory tract, pancreas and urinary bladder
BRUCE A. MACHER, PH.D., *Assistant Professor of Pharmaceutical Chemistry*, University of California, San Francisco.	Chemistry and biology of complex carbohydrates
RICHARD A. MARKHAM, M.D., *Assistant Professor of Medicine and of Microbiology and Immunology*, The Jewish Hospital of St. Louis, St. Louis.	T cell-mediated immunity to *Pseudomonas aeruginosa*
ALAN C. McLAUGHLIN, PH.D., *Lecturer in Biochemistry/Biophysics*, University of Pennsylvania School of Medicine, Philadelphia.	Interaction of divalent cations with model and biological membranes
FERID MURAD, M.D., PH.D., *Professor of Medicine and Pharmacology*, Stanford University, and *Chief of Medicine*, Palo Alto V.A. Hospital, Stanford, CA.	Mechanism of nitric oxide activation of guanylate cyclase Role of cyclic GMP in smooth muscle relaxation
CHRISTOPHER MURLAS, M.D., *Assistant Professor of Medicine*, University of California School of Medicine, Irvine.	Airway muscle abnormalities in bronchial hyperreactivity
JAY A. NADEL, M.D., *Professor of Medicine, Physiology and Radiology*, Cardiovascular Research Institute, University of California, San Francisco.	Mechanisms of airway hyperreactivity
DONALD J. NELSON, PH.D., *Associate Professor of Chemistry*, Clark University, Worcester, MA.	Interaction of cholinergic ligands with genetic variants of the acetylcholine receptor
STAFAN NIEWIAROWSKI, M.D., PH.D., *Professor of Physiology*, Thrombosis Research Center, Temple University School of Medicine, Philadelphia.	Platelet interaction with fibrinogen and its significance in hemostasis
F. WILLIAM ORR, M.D., *Associate Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Role of local factors in pulmonary metastasis
BENDICHT U. PAULI, D.V.M., *Associate Professor of Pathology*, Rush Presbyterian-St. Lukes Medical Center, Chicago.	Local regulation of tumor invasion by host-derived proteinase inhibitors
BORIS M. PETERLIN, M.D., *Assistant Professor of Medicine*, Section of Rheumatology-Clinical Immunology, University of California School of Medicine, San Francisco.	Biology and molecular biology of the differentiation of a human monocytoid cell line

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
DENNIS R. PETERSEN, PH.D., *Associate Professor of Pharmacology*, University of Colorado School of Pharmacy, Boulder.	Implementation of the isolated perfused liver to study nicotine metabolism and metabolic interactions
JULIA M. POLAK, D.SC., M.D., *Senior Lecturer in Histopathology*, Royal Postgraduate Medical School, Hammersmith Hospital, London.	Investigation of the role of regulatory peptides in human lung disease
WILLIAM A. PRYOR, PH.D., *Boyd Professor of Chemistry*, Louisiana State University, Baton Rouge.	Free radical chemistry of cigarette smoke
MICHAEL S. RABSON, PH.D., *Research Scientist, Department of Epidemiology and Public Health*, Yale Medical School, New Haven, CT.	Analysis of sequences required for bovine papilloma virus transformation and autonomous plasmid replication
TIMOTHY J. REGAN, M.D., *Professor of Medicine; Director, Division of Cardiovascular Diseases*, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark.	Susceptibility of arrhythmias and catecholamine metabolism in chronic smoking animals
JOHN E. REPINE, M.D., *Assistant Director, Webb-Waring Lung Institute; Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	Basic mechanisms of lung injury from inhaled oxidants
ROBERT RESNICK, M.D., *Associate Professor of Reproductive Medicine*, University of California Medical Center, San Diego.	The effect of nicotine on uterine and fetal cardiovascular hemodynamics
HERBERT Y. REYNOLDS, M.D., *Professor of Medicine; Head, Pulmonary Section*, Yale University School of Medicine, New Haven, CT.	Respiratory secretions in pulmonary carcinoma: secretory component of immunoglobulin-A as an early marker of epithelial dysfunction
	Markers of epithelial cell dysfunction in respiratory secretions of smokers
VIRGINIA L. RICHMOND, PH.D., *Research Associate*, Pacific Northwest Research Foundation, Seattle.	Elastic fiber microfibrillar protein structure
EUGENE ROBERTS, PH.D., *Research Scientist*, City of Hope Research Institute, Duarte, CA.	Effects of nicotine on the cerebrovasculature *in vitro*
PETER M. ROSS, PH.D., *Research Associate*, The Rockefeller University, New York.	DNA damage and selective maintenance of animal genes
UNA S. RYAN, PH.D., *Research Professor of Medicine*, University of Miami School of Medicine, Miami, FL.	Interactions of hormones with cells of the pulmonary vascular wall
B. V. RAMA SASTRY, D.SC., PH.D., *Professor of Pharmacology*, Vanderbilt University School of Medicine, Nashville, TN.	Influence of nicotine on the release of acetylcholine in the human placenta and its implications on the fetal growth
ISHAIAHU SCHECHTER, PH.D., *Senior Lecturer in Biochemistry*, The George S. Wise Faculty for Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Effect of thiols and disulfides on cholesterol metabolism

PRINCIPAL INVESTIGATOR OR INSTITUTION
ROBERT J. SKLAREW, PH *Associate Professor of Pa* York University Resea Goldwater Memorial Hospi Island, New York.
DENNIS M. SMITH, PH.D., *fessor of Biological Scienc* College, Wellesley, MA.
STEVEN S. SMITH, PH.D., *search Scientist*, City of Hop stitute, Duarte, CA.
TIMOTHY A. SPRINGER, PH *Professor of Pathology; Chi of Membrane Immunocher* Farber Cancer Institute, Bos
ERIC J. STANBRIDGE, PH *Professor of Microbiology*, California, Irvine.
NORMAN C. STAUB, M.D *Physiology*, Cardiovascular tute, University of Califor cisco.
DANIEL STEINBERG, M.D *fessor of Medicine; Head, Du bolic Disease*, The Universit at San Diego, La Jolla.
THOMAS P. STOSSEL, M.D *cal Oncology Unit*, Massach Hospital, Boston.
D. LANSING TAYLOR, PH.D *Biology*, Carnegie-Mellor Pittsburgh.
JOSEPH CHARLES TAYLOR *ciate Research Scientist*, Ci search Institute, Duarte, CA
JOHN A. THOMPSON, PH *Professor of Pharmaceutic* University of Colorado Sc macy, Boulder.
JAMES TRAVIS, PH.D., *Pr chemistry*, The University Athens.
EMIL R. UNANUE, M.D. *Professor of Immunopatho* Medical School, Boston.
STEPHEN F. VATNER, M *Professor of Medicine*, H School, New England Regio search Center, Southboro, *in Medicine*, Peter Bent Bri Boston.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ROBERT J. SKLAREW, PH.D., *Research Associate Professor of Pathology*, New York University Research Service, Goldwater Memorial Hospital, Roosevelt Island, New York.	Cytokinetics of heteroploid subpopulations by imaging
DENNIS M. SMITH, PH.D., *Assistant Professor of Biological Sciences*, Wellesley College, Wellesley, MA.	Autonomic control of pulmonary surfactant in the adult lung
STEVEN S. SMITH, PH.D., *Assistant Research Scientist*, City of Hope Research Institute, Duarte, CA.	Selectivity of DNA methylation in normal and oncogenically transformed cells
TIMOTHY A. SPRINGER, PH.D., *Assistant Professor of Pathology; Chief, Laboratory of Membrane Immunochemistry*, Sidney Farber Cancer Institute, Boston.	Studies of macrophage subpopulations and differentiation using monoclonal antibodies
ERIC J. STANBRIDGE, PH.D., *Associate Professor of Microbiology*, University of California, Irvine.	Transfer of specific individual human chromosomes to recipient cells
NORMAN C. STAUB, M.D., *Professor of Physiology*, Cardiovascular Research Institute, University of California, San Francisco.	Alveolar-airway barrier permeability to liquid and macromolecules in dog and sheep lung
DANIEL STEINBERG, M.D., PH.D., *Professor of Medicine; Head, Division of Metabolic Disease*, The University of California at San Diego, La Jolla.	Arterial degradation of low density lipoproteins *in vivo*
THOMAS P. STOSSEL, M.D., *Chief, Medical Oncology Unit*, Massachusetts General Hospital, Boston.	Functional anatomy of the lung macrophage
D. LANSING TAYLOR, PH.D., *Professor of Biology*, Carnegie-Mellon University, Pittsburgh.	Chemotaxis of macrophages
JOSEPH CHARLES TAYLOR, PH.D., *Associate Research Scientist*, City of Hope Research Institute, Duarte, CA.	Ceruloplasmin abnormality in chronic obstructive pulmonary disease
JOHN A. THOMPSON, PH.D., *Associate Professor of Pharmaceutical Chemistry*, University of Colorado School of Pharmacy, Boulder.	Chromatographic separation and comparative metabolism of d- and l-nicotine
JAMES TRAVIS, PH.D., *Professor of Biochemistry*, The University of Georgia, Athens.	Proteolytic enzymes and inhibitors in emphysema
EMIL R. UNANUE, M.D., *Mallinckrodt Professor of Immunopathology*, Harvard Medical School, Boston.	Physiopathology of normal and activated macrophages
STEPHEN F. VATNER, M.D., *Associate Professor of Medicine*, Harvard Medical School, New England Regional primate Research Center, Southboro, MA.; *Associate in Medicine*, Peter Bent Brigham Hospital, Boston.	Direct effects of nicotine on brain circulation

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
EVELYN WALDSTEIN, PH.D., *Senior Lecturer*, Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel.	Repair activities for O^6-methylguanine DNA adducts in human lymphocytes of smokers *vs.* nonsmokers
PETER N. WALSH, PH.D., *Professor of Medicine*, Temple University School of Medicine, Philadephia.	Interaction of platelets with coagulation factors IX and X
PETER A. WARD, M.D., *Professor and Chairman, Department of Pathology*, The University of Michigan, Ann Arbor.	Oxygen-derived free radicals, immune complexes and tissue injury
GEORGE WEINBAUM, PH.D., *Assistant Chairman for Research, Department of Medicine*, The Graduate Hospital, Philadelphia.	The role of peptide methionine sulfoxide reductase in human lungs: a possible defense against protein oxidation and elastin degradation in smokers
	Bronchioalveolar lavage of human smokers and nonsmokers: studies on cell chemotaxis, enzyme release and cellular ultrastructure
I. BERNARD WEINSTEIN, M.D., *Professor of Medicine and Environmental Sciences*, Columbia University, New York.	Development of monoclonal antibodies to carcinogen-DNA adducts
SAMUEL B. WEISS, PH.D., *Professor of Biochemistry and Microbiology*, The University of Chicago, Chicago.	Sequence modifications in viral DNA by benzo(α)pyrene metabolites
SIGMUND A. WEITZMAN, M.D., *Assistant Professor of Medicine*, Hematology—Oncology Unit, Massachusetts General Hospital, Boston.	Studies of phagocyte-induced mutation
ÅKE WENNMALM, M.D., *Associate Professor of Clinical Physiology at Karolinska Institute*, Huddinge Hospital, Huddinge, Sweden	Nicotine as inhibitor of prostaglandin formation: localization of the inhibitory step and characterization of the cardiovascular implications
JOHN T. WILSON, PH.D., *Assistant Professor of Cell and Molecular Biology*, Medical College of Georgia, Augusta.	The isolation and expression of human α-1-antitrypsin gene sequences through molecular cloning
ALVIN L. WINTERS, PH.D., *Assistant Professor of Microbiology and Biochemistry*, The University of Alabama, University.	Interferon induction in the human population, effects of smoking, chronic disease and cancer
STANLEY YACHNIN, M.D., *Professor of Medicine and Chief, Section of Hematology/Oncology*, The University of Chicago Medical Center, Chicago.	Models for the pathogenesis of atherosclerosis: A) biological effects of oxygenated sterol compounds; B) mevalonic acid and cholesterol biosynthesis and the biosynthesis and regulation of cell growth

Con

Following is a list of th
have been completed prio
individuals names are dece
at the time the work was c

MARIO D. ACETO, PH.D., *fessor of Pharmacology*, M of Virginia, Virginia Comm versity, Richmond

CLARENCE M. AGRESS, M *Clinical Professor of Medic* of California Medical Angeles.

ANTHONY A. ALBANESE, *tor of Laboratories*, The Bu tion Center, White Plains,

ANTHONY P. AMAROSE, F *tor in Obstetrics and Gynec* bany Medical College of Un Albany, NY.

E. T. ANGELAKOS, M.D., *sor of Physiology*, Bost School of Medicine, Bosto

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STEPHEN M. AYRES, M *Cardiopulmonary Laborat* cent's Hospital, New York

OSCAR J. BALCHUM, PH *Professor of Medicine*, Southern California Schoo Los Angeles.

Completed Projects

Following is a list of the principal investigators, or institutions, of projects that have been completed prior to the period covered in this Report. Several of the individuals names are deceased. The titles and affiliations listed are those in effect at the time the work was completed.

MARIO D. ACETO, PH.D., *Associate Professor of Pharmacology*, Medical College of Virginia, Virginia Commonwealth University, Richmond.

CLARENCE M. AGRESS, M.D., *Associate Clinical Professor of Medicine*, University of California Medical Center, Los Angeles.

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ANTHONY P. AMAROSE, PH.D., *Instructor in Obstetrics and Gynecology*, The Albany Medical College of Union University, Albany, NY.

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D. MURRAY ANGEVINE, M.D., University of Wisconsin School of Medicine, Madison.

JOSEPH C. ARCOS, D.SC., *Professor of Medicine*, Tulane University School of Medicine, New Orleans.

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OSCAR J. BALCHUM, PH.D., *Hastings Professor of Medicine*, University of Southern California School of Medicine, Los Angeles.

FREDERIK B. BANG, M.D., *Professor and Chairman, Department of Pathobiology*, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.

A. CLIFFORD BARGER, M.D., *Robert Henry Pfeiffer Professor of Physiology*, Harvard Medical School, Boston.

BRODA O. BARNES, M.D., PH.D., *Professor (Affiliate) of Physiology*, Colorado State University, Fort Collins.

FREDERICK W. BARNES, JR., M.D., *Associate Professor of Medicine*, The Johns Hopkins University School of Medicine, Baltimore.

T. C. BARNES, D.SC., *Research Scientist*, Philadelphia State Hospital, Philadelphia.

CARL G. BECKER, M.D., *Associate Professor of Pathology*, Cornell University Medical College, New York.

R. FREDERICK BECKER, PH.D., *Associate Professor of Anatomy and Director*, Laboratory of Perinatal Science, Duke University Medical Center, Durham, NC.

RALPH S. BECKER, PH.D., *Professor of Chemistry*, University of Houston, Houston.

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